



ANAIS DO 49º CONGRESSO DA SOCIEDADE BRASILEIRA DE IMUNOLOGIA (SBI)

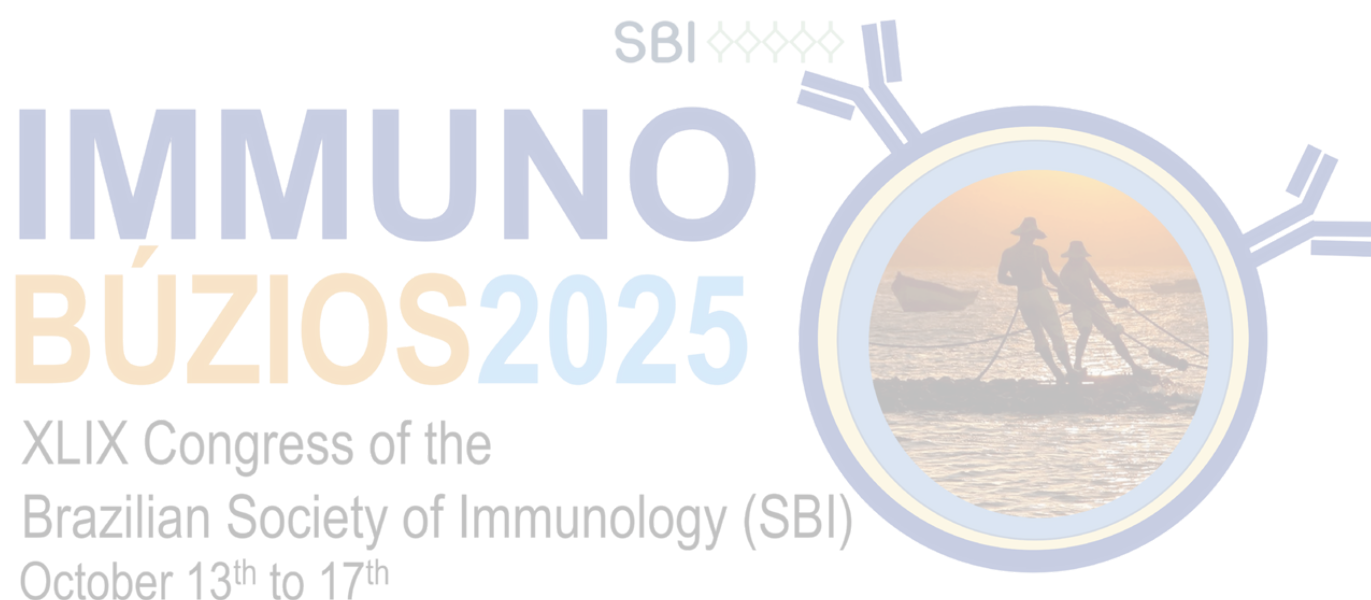
SBI 
IMMUNO
BÚZIOS2025

XLIX Congress of the
Brazilian Society of Immunology (SBI)
October 13th to 17th



XLIX CONGRESS OF BRAZILIAN SOCIETY OF IMMUNOLOGY

PROGRAM



#ImmunoBúzios2025



October 12th - Sunday

PRE-COURSE - UNDERSTANDING THE BIOLOGY OF REGULATORY T CELLS

02:30 PM to 06:20 PM
ROOM TUCUNS

Chairs: Maria Bellio (Federal University of Rio de Janeiro - Brazil) & José Carlos Alves-Filho (University of São Paulo - Ribeirão Preto - Brazil)

02:30 PM - 02:40 PM Opening

02:40 PM - 03:40 PM **Regulation of Regulatory T Cell Function by Foxp3**

Steven Ziegler - Benaroya Research Institute - United States of America

03:40 PM - 04:40 PM **Control of Regulatory T Cell Differentiation and Function**

José Carlos Alves-Filho - University of São Paulo - Ribeirão Preto - Brazil

04:50 PM - 05:20 PM **Coffee break**

05:20 PM - 06:20 PM **Metabolic checkpoints for manipulating Treg function**

Tim Sparwasser - University Medical Center of Mainz - Germany

PRE-CONGRESS - SATELLITE MEETING: VACCINES

03:00 PM to 07:00 PM
ROOM TARTARUGA

03:00 PM - 03:10 PM **Opening** Silvia Beatriz Boscardin - University of São Paulo - Brazil

03:10 PM - 04:00 PM **Opening Lecture: Vaccine Research at the Oswaldo Cruz Foundation (FIOCRUZ)**

Alda Maria da Cruz - Fiocruz - Rio de Janeiro - Brazil

04:00 PM - 06:10 PM **Round Table 1: mRNA Vaccination Technology**

Chair: Herbert Leonel de Matos Guedes - Federal University of Rio de Janeiro - Brazil

04:00 PM - 04:25 PM **FIOCRUZ RNA Platform: A Strategic Engine for Regional Vaccine Development**

Patrícia C. da Costa Neves - Bio-Manguinhos/Fiocruz - Brazil

04:25 PM - 04:50 PM **Nforce toolbox and rethinking the paradigm of RNA therapeutic development**

Akm Ashiqul Haque - Quantoom Bioscience - Belgium

04:50 PM - 05:20 PM **Coffee break**

05:20 PM - 05:45 PM **Testing different platforms for vaccine development against Chagas disease**

Santuza Maria Ribeiro Teixeira - Federal University of Minas Gerais - Brazil

05:45 PM - 06:10 PM **Vaccine development against leishmaniasis**

Herbert Leonel De Matos Guedes - Federal University of Rio De Janeiro - Brazil

06:10 PM - 07:00 PM **Conference: Systems Vaccinology and Big Data in Vaccine Development**

Helder Imoto Nakaya - Albert Einstein Hospital - Brazil

Chair: Santuza Maria Ribeiro Teixeira - Federal University of Minas Gerais - Brazil

October 13th, Monday

CAPACITAÇÃO MULTIPROFISSIONAL EM HANSENÍASE (TURMA 1)/ MULTIDISCIPLINARY TRAINING IN HANSEN'S DISEASE (CLASS 1)

09:00 AM to 12:00 PM

ROOM GERIBÁ

Coordinator: Roberta Olmo Pinheiro - Fiocruz - Brazil

PRE-CONGRESS - SATELLITE MEETING: VACCINES

09:00 AM to 11:30 AM

ROOM TARTARUGA

09:00 AM - 10:40 AM **Round Table 2: Vaccines for Neglected Tropical Diseases (Vaccines the World Needs)**

Chair: Herbert Leonel de Matos Guedes - Federal University of Rio de Janeiro - Brazil

09:00 AM - 09:25 AM **Development of the Sm14 -FABP Multivalent Anti Helminth Vaccine**

Miriam Tendler - IOC/FIOCRUZ - Brazil

09:25 AM - 09:50 AM **Plasmodium vivax malaria vaccine**

Irene da Silva Soares - University of São Paulo/ INCT-V - Brazil

09:50 AM - 10:15 AM **Toward a Prophylactic and Therapeutic Chagas Disease Vaccine: How Close Are We?**

Emilio Luis Malchiodi - University of Buenos Aires - Argentina

10:15 AM - 10:40 AM **A Chimeric Recombinant Vaccine for Visceral Leishmaniasis**

Ana Paula Fernandes - Federal University of Minas Gerais - Brazil

10:40 AM - 11:30 AM **Conference: Clinical Development of Butantan-DV, a single-dose tetravalent dengue vaccine**

Érique José Farias Peixoto de Miranda - Butantan Institute - Brazil

Chair: Herbert Leonel de Matos Guedes - Federal University of Rio de Janeiro - BRAZIL

PRE-COURSE - UNDERSTANDING THE BIOLOGY OF REGULATORY T CELLS

09:00 AM to 12:00 PM

ROOM TUCUNS

Chair: José Carlos Alves-Filho - University of São Paulo - Ribeirão Preto - Brazil

09:00 AM - 10:00 AM **Tailoring Treg Function in Th1 Immunity**

Nicole Christine Joller - University of Zurich - Switzerland

10:00 AM - 11:00 AM **Peripheral homeostasis of CD4⁺Foxp3⁺ regulatory T cells**

Fábio Canto - Fluminense Federal University - Brazil

11:00 AM - 12:00 PM **Harnessing Regulatory T Cells for the Development of Novel Therapies in Immune-Mediated Diseases: Insights from IBD**

Raphael S. Peres - University of São Paulo-Ribeirão Preto - Brazil

Sessão Especial: Ensinando Imunologia nas Escolas/

Special Session: Teaching Immunology to Kids

09:30 AM to 12:00 PM

ROOM FERRADURA & JOÃO FERNANDES

WORKSHOP: Tudo que você sempre quis saber sobre imunologia/ EVERYTHING YOU ALWAYS WANTED TO KNOW ABOUT IMMUNOLOGY

Chair: Robson Coutinho-Silva - Federal University of Rio de Janeiro - Brazil

Verônica Coelho - University of São Paulo - Brazil

Viviane Abreu de Andrade - Federal Center for Technological Education Celso Suckow da Fonseca - Brazil

Raquel Cavalcanti De Albuquerque - Maricá School Network - Brazil

Valéria Borges - Fiocruz-BA - Brazil

09:30 AM - 12:00 PM Evento na Escola/ Activity at the School Municipal Antônio Alípio da Silva

Discutindo Vacinas e Saúde na Escola/ Discussing Vaccines and Health at the School

Claudia Ida Brodskyn - Fiocruz - Brazil

LUNCH & FREE TIME

12:00 PM to 01:00 PM

CAPACITAÇÃO MULTIPROFISSIONAL EM HANSENÍASE (TURMA 2)/

MULTIDISCIPLINARY TRAINING IN HANSEN'S DISEASE (CLASS 2)

01:00 PM to 04:00 PM

ROOM GERIBÁ

Coordinator: Roberta Olmo Pinheiro - Fiocruz - Brazil

PRE-CONGRESS - SATELLITE MEETING: VACCINES

01:00 PM to 03:30 PM

ROOM TARTARUGA

Chair: Silvia Beatriz Boscardin - University of São Paulo - Brazil

01:00 PM - 02:40 PM Round Table 3: Immunological Responses to Vaccination

01:00 PM - 01:25 PM Understanding the basic biology of plasma cells as a means toward developing better vaccines

Renan V. H. de Carvalho - The Scripps Research Institute - United States of America

01:25 PM - 01:50 PM Targeting Protective B cell Epitopes for Therapeutic Antibodies against Mpox

Camila Henriques Coelho - Icahn School of Medicine at Mount Sinai - United States of America

01:50 PM - 02:15 PM Past Encounters, Future Protection: Harnessing Immune Imprinting for Improved Vaccines

Carolina Lucas - Yale School of Medicine - United States of America

02:15 PM - 02:40 PM Leveraging T cell immunity for next generation vaccines: lessons from malaria research

Daniel Fernandez Ruiz - University of New South Wales - Australia

02:40 PM - 03:30 PM Closing Conference: Development of an Intranasal Vaccine Against SARS-CoV-2

Edecio Cunha Neto - University of São Paulo - BRAZIL

Sessão Especial: Ensinando Imunologia nas Escolas/ Special Session: Teaching Immunology to Kids

02:00 PM to 04:00 PM

ROOM 1 - FERRADURA & JOÃO FERNANDES

Pesquisa em Ensino de Ciências da Saúde e Divulgação Científica/ Symposium: Research in Health Sciences Education and Science Communication

Chairs: Robson Coutinho-Silva - Federal University of Rio de Janeiro - Brazil & Raquel Cavalcanti de Albuquerque - Maricá School Network - Brazil

02:00 PM - 02:40 PM Experiências na Ciência em Educação e Divulgação Científica da Fiocruz Bahia/ Science Education and Outreach in the Health Sciences: The Fiocruz Bahia Experience

Valéria Borges - Fiocruz-BA - Brazil

02:40 PM - 03:20 PM Projeto Imunologia nas Escolas: aprendendo com uma experiência de 10 anos/ Immunology in Schools Project: learning from a 10 - year experience

Verônica Coelho - University of São Paulo - Brazil

03:20 PM - 04:00 PM Alguns aspectos acerca da complexidade do ensino e da aprendizagem da Imunologia/ Some aspects about the complexity of teaching and learning Immunology

Viviane Abreu de Andrade - Federal Center for Technological Education Celso Suckow da Fonseca - Brazil

INTERMISSION FOR SESSION TRANSITION

04:00 PM to 05:00 PM

OPENING CEREMONY - SBI LIFETIME ACHIEVEMENT AWARD IN IMMUNOLOGY

05:00 PM to 06:00 PM

PLENARY SESSION

Chair: Marcello Barcinski - Federal University of Rio de Janeiro - Brazil

OPENING CONFERENCE - FOCUSING IMMUNE RESPONSES ON CONSERVED AND IMMUNOGENIC REGIONS OF MICROBES OF PANDEMIC CONCERN

06:00 PM to 07:00 PM

PLENARY SESSION

Alessandro Sette - La Jolla Institute for Immunology - United States of America

Chair: Edecio Cunha Neto - University of São Paulo - Brazil

COCKTAIL

07:00 PM to 09:00 PM

1ST FLOOR - POOL AREA

October 14th, Tuesday

BRIGHT SPARKS 1 - B CELLS AND GERMINATIVE CENTERS

08:00 AM to 08:50 AM

ROOM FERRADURA & JOÃO FERNANDES

Chair: Tatiani Uceli Maioli - Federal University of Minas Gerais - Brazil

08:00 AM - 08:10 AM **Tertiary Lymphoid Structures Drive Germinal Center B Cell Maturation and Tumor-reactive IgG in Renal Cancer**

Raylane Adrielle Gonçalves Cambui - Brazil (MT)

08:12 AM - 08:22 AM **FOXP3+ CD4 T follicular cells potentially restrict tertiary lymphoid structure and germinal center development in endometrial cancer patients**

Maria Luisa Marques Pierre - Brazil

08:24 AM - 08:34 AM **Fluzone High-Dose Vaccine Boosts HA-Specific Memory B Cells via Coordinated Activation of Antibody-Secreting Cells and HA-Specific Th1 and cTfh in Older Adults**

Vanessa Silva Moraes - Brazil

08:36 AM - 08:46 AM **Uncovering Human Mucosal Immunity: Functional Profiling of Nasal B Cells**

Bianca Klakfe Beck - Brazil

BD MINI COURSE - IDENTIFY: THE POWER OF CHOICE

08:00 AM to 08:50 AM

ROOM GERIBÁ & TARTARUGA

Guilherme Ferreira de Brito Evangelista - Brazil

COURSE - UNIVERSE OF EXTRACELLULAR VESICLES (EVS)

08:00 AM to 08:50 AM

ROOM TUCUNS

Historical Overview and Definition of Extracellular Vesicles (EVs)

Ana Claudia Trocoli Torrecilhas - UNIFESP - Brazil

- a. Overview of EVs.
- b. Historical events in EVs research and advancements in technology.
- c. Definition and classification of EVs, classification criteria based on MISEV 2023 (Internacional Society for Extracellular Vesicles (ISEV) guideline).
- d. Functions and biogenesis of EVs.

SYMPOSIUM 01 - INNATE IMMUNITY I

09:00 AM to 10:40 AM

ROOM FERRADURA & JOÃO FERNANDES

Chair: Karina Ramalho Bortoluci - UNIFESP - Brazil

09:00 AM - 09:25 AM **Cellular and molecular regulation of inflammation**

Ivan Zanoni - Harvard Medical School - United States of America

09:25 AM - 09:50 AM **Cell clearance and tissue repair**

Sophia Maschalidi - VIB-UGent Center for Inflammation Research - Belgium

09:50 AM - 10:15 AM **Immune complexes as triggers of long Covid-19**

Caroline Furtado Junqueira - Institute for Research in Biomedicine - Switzerland

10:15 AM - 10:27 AM **Short talk 1: mitoKATP Channel Suppression Drives LPS Tolerance via ROS-Mediated HIF-1 α /NF- κ B and Mitochondrial Hyperfission|** Presenter: Lincon Felipe Lima Silva

10:28 AM - 10:40 AM **Short Talk 2: Macrophage AHR Deficiency Drives Obesity-Associated Metabolic Dysfunction |** Presenter: Bianca Gazieri Castelucci

SYMPOSIUM 02 - IMMUNE RESPONSES IN CANCER

09:00 AM to 10:40 AM

ROOM GERIBÁ & TARTARUGA

Chair: João Viola - INCA - Brazil

09:00 AM - 09:25 AM **Tracking T cell recruitment to colorectal cancer**

Daniel Mucida - Rockefeller University - United States of America

09:25 AM - 09:50 AM **The natural repertoire as a source of novel anti-checkpoint antibodies**

Cristina Bonorino - Federal University of Health Sciences of Porto Alegre - Brazil

09:50 AM - 10:15 AM **Immune System Networks as Determinants of Therapy Response in Cancer**

Kenneth J. Gollob - Albert Einstein Hospital - Brazil

10:15 AM - 10:40 AM **Fat Matters: The Impact of Obesity and Adipose Tissue on Cancer Progression and Therapy**

Kelly Grace Magalhães - University of Brasilia - Brazil

SYMPOSIUM 03 - T CELL TISSUE RESIDENCY

09:00 AM to 10:40 AM

ROOM TUCUNS

Chair: Henrique Borges da Silva - Mayo Clinic - United States of America

09:00 AM - 09:25 AM Spatial Instructions of Tissue T Cell Immunity

Miguel Reina-Campos - La Jolla Institute for Immunology - United States of America

09:25 AM - 09:50 AM Resident T cells and post viral tissue pathology

Jie Sun - University of Virginia - United States of America

09:50 AM - 10:15 AM Blood stage malaria undermines liver TRM-mediated immunity in select vaccine contexts

Daniel Fernandez-Ruiz - University of New South Wales - Australia

10:15 AM - 10:27 AM Short talk 1: The extracellular ATP sensor P2RX7 sustains the metabolic fitness of tissue-resident CD4⁺ T helper cells and promotes protective immunity against influenza reinfection | Presenter: Igor Santiago-Carvalho

10:28 AM - 10:40 AM Short talk 2: Circulating and Tissue-Resident Memory T Cells Display Distinct Differentiation and Cytotoxicity Profiles in Localized Cutaneous Leishmaniasis | Presenter: Renan Garcia de Moura

COFFEE BREAK - INTERMISSION FOR SESSION TRANSITION

10:40 AM to 11:10 AM

KEYNOTE CONFERENCE 2 - THE BIOLOGY BEHIND PD-1 BLOCKADE

11:10 AM to 12:00 PM

PLENARY SESSION

Arlene H. Sharpe - Harvard Medical School - United States of America

Chair: João P. Viola - INCA - Brazil

SPONSORED SYMPOSIUM - THERMOFISHER

12:00 PM to 12:30 PM

PLENARY SESSION

Spectral Revolution: The New Era of Microscopy, Cytometry, and Cell Sorting

Lucas Rizzo

SPONSORED SYMPOSIUM - DBR / CYTEK

12:35 PM to 01:05 PM

PLENARY SESSION

Turning Light Into Insight: Advancing Flow Cytometry Data Quality With Cytex's High-Resolution Spectral Technology

Maria C. Jaimes, MD - United States of America

INTERMISSION FOR SESSION TRANSITION

01:05 PM to 01:35 PM

BRIGHT SPARKS 2 - INFECTIOUS DISEASES

01:40 PM to 02:30 PM

ROOM FERRADURA

Chair: Momtchilo Russo - University of São Paulo - Brazil

01:40 PM - 01:50 PM **Annexin A1 is crucial during murine model of T. cruzi infection, preventing tissue damage, immune dysregulation and gut microbiota imbalance**

Fernando Bento Rodrigues Oliveira - Brazil

01:52 PM - 02:02 PM **CCR4-Driven regulatory T cells migration shapes the outcome of Leishmania Infection**

Giovana Gomes Chagas - Brazil

02:04 PM - 02:14 PM **FABP-2 plasmatic levels and its relationship with cytokines during Plasmodium infection in individuals living in the Brazilian amazon**

Cinthia Magalhães Rodolphi - Brazil

02:16 PM - 02:26 PM **Ly6C^{high} Macrophages as the Main Inflammatory Cell in the Lungs of C3H/HeJ Mice in a Sublethal Model for Leptospirosis**

Ana Carolina Mikejevs Lorga - Brazil

BRIGHT SPARKS 3 - NEUROIMMUNOLOGY

01:40 PM to 02:30 PM

ROOM JOÃO FERNANDES

Chair: Patrícia Torres Bozza - Fiocruz - Rio de Janeiro - Brazil

01:40 PM - 01:50 PM **Association study of immunogenetic factors with anxiety and depression symptoms in Brazilian children**

Alana Conceição Maia Lessa - Brazil

01:52 PM - 02:02 PM **Neuroimmune and gastrointestinal correlates of shrimp allergy in a mouse model**

Camila Mattos Andrade - Brazil

02:04 PM - 02:14 PM **Infection-induced disruption of the cholinergic pathway impacts mesenteric immune tone by inducing regulatory T cell reprogramming**

Laura Barbosa da Conceição - Brazil

02:16 PM - 02:26 PM **Pulmonary allergic inflammation increases microglia activation associated with depressive behavior**

Ualter Guilherme Cipriano Rosa - Brazil

BRIGHT SPARKS 4 - T CELL BIOLOGY

01:40 PM to 02:30 PM

ROOM GERIBÁ

Chair: Renata de Meirelles Santos Pereira - Federal University of Rio de Janeiro - Brazil

01:40 PM - 01:50 PM **Characterization of the Involvement of the Transcriptional Cofactor IRF2BP2 in CD8 T Cell Proliferation**

Bárbara Van Steen Proner Ramos - Brazil

01:52 PM - 02:02 PM **The role of DMT1 iron transporter in activation and differentiation of Th1 lymphocytes in Mycobacterium tuberculosis infection**

Ana Clara Matoso Montuori de Andrade - Brazil

02:04 PM - 02:14 PM **Role of transcriptional co-factor IRF2BP2 in activation and differentiation of CD8 T cells**

Barbara Lucia Morais de Oliveira Vieira - Brazil

02:16 PM - 02:26 PM **Infection-induced regulatory T cell dysfunction in the gut restricts colorectal tumorigenesis**

Leonardo Mandu Gonçalves - Brazil

BRIGHT SPARKS 5 - CLINICAL IMMUNOLOGY

01:40 PM to 02:30 PM

ROOM TARTARUGA

Chair: Kelly Grace Magalhães - University of Brasilia - Brazil

01:40 PM - 01:50 PM **Cancer vaccines synergize with adoptively transferred T lymphocytes against solid tumors in a CXCR3-dependent manner**

Vinnycius Pereira Almeida - Brazil

01:52 PM - 02:02 PM **Hypertension is associated with accelerated immunosenescence**

Lícia Torres - Brazil

02:04 PM - 02:14 PM **Innate-like T Cell Signatures in Oral Squamous Cell Carcinoma Reveal Links to Lymph Node Invasion and Disease Progression**

Flávio Pignataro Oshiro - Brazil

02:16 PM - 02:26 PM **Immune signatures of wild-type yellow fever virus infection: insights from the 2016–2018 Brazilian outbreak**

Andreza Parreiras Gonçalves - Brazil

BRIGHT SPARKS 6 - LEUKOCYTE BIOLOGY

01:40 PM to 02:50 PM

ROOM TUCUNS

Chair: Gustavo Menezes - Federal University of Minas Gerais - Brazil

01:40 PM - 01:50 PM **GCN2 controls ZAK α signaling to limit inflammatory activation in macrophages**

Rodrigo Dias Requiao - Brazil

01:52 PM - 02:02 PM **Immunometabolic Reprogramming of Neutrophils in Sepsis: A Proteomic and Functional Perspective**

Mônica Bragança Sousa - Brazil

02:04 PM - 02:14 PM **Distribution and phenotypic changes of B cells in the mouse brain from development to aging**

Danillo Pereira Dantas - Brazil

02:16 PM - 02:26 PM **Surveillance of SARS-CoV-2 Immunogenicity and Virus-Specific CD8⁺ T Cells Reveal Fragility of Immunodominant HLA-A*02-Restricted Epitopes that Activate CD8⁺ T Cells**

Ágata Lopes Ribeiro - Brazil

02:28 PM - 02:38 PM **DRP1 is necessary for macrophage IL-6 production and BAT adaptation during cold exposure**

Marcelo Rodrigues Berçot - Brazil

02:40 PM - 02:50 PM **B-lymphocyte-intrinsic MyD88 signaling controls the intestinal TREG–IgA–microbiota axis under the steady-state**

Isabella Nogueira da Silva Alves - Brazil

Intermission for Session Transition

02:30 PM to 03:00 PM

SYMPOSIUM 04 - TREGS & IMMUNOREGULATION

03:00 PM to 04:40 PM

ROOM FERRADURA & JOÃO FERNANDES

Chair: José Carlos Alves-Filho - University of São Paulo - Ribeirão Preto - Brazil

03:00 PM - 03:25 PM **Tailoring Treg Function in Th1 Immunity**

Nicole Joller - University of Zurich - Switzerland

03:25 PM - 03:50 PM **Regulation of Regulatory T Cell Function by Foxp3**

Steven Ziegler - Benaroya Research Institute - United States of America

03:50 PM - 04:15 PM **B-cell-driven control of gut-associated CD4⁺Foxp3⁺ Treg cell homeostasis**

Fábio Canto - Fluminense Federal University - Brazil

04:15 PM - 04:40 PM **Regulation of Treg stability by metabolism and posttranslational protein modifications**

Luciana Elda Berod - University of Mainz - Germany

SYMPOSIUM 05 - B CELLS & ANTIBODIES I

03:00 PM to 04:40 PM

ROOM GERIBÁ & TARTARUGA

Chair: Alberto Nóbrega - Federal University of Rio de Janeiro - Brazil

03:00 PM - 03:25 PM **Improving *P. falciparum* circumsporozoite protein vaccine responses**

Hedda Wardemann - German Cancer Research Center - Germany

03:25 PM - 03:50 PM **Antibodies and autoantibodies during infection**

Davide Robbiani - Institute for Research in Biomedicine - Switzerland

03:50 PM - 04:15 PM **Regulation of the germinal center B cell response to vaccination**

Ali H. Ellebedy - Washington University School of Medicine - United States of America

04:15 PM - 04:40 PM **Extrinsic factors dictating plasma cell longevity**

Renan V. H. de Carvalho - The Scripps Research Institute - United States of America

SYMPOSIUM 06 - AUTOPHAGY IN IMMUNITY

03:00 PM to 04:40 PM

ROOM TUCUNS

Chair: Leonardo Travassos Corrêa - Federal University of Rio de Janeiro - Brazil

03:00 PM - 03:25 PM Canonical and noncanonical roles for autophagy proteins in regulating inflammation

Christina Stallings - Washington University - United States of America

03:25 PM - 03:50 PM Exploring the role of non-canonical autophagy in regulating cellular stress responses

Oliver Florey - Babraham Institute - United Kingdom

03:50 PM - 04:15 PM SPATA5 loss of function drives STING activation and type 1 interferon production

Dana Philpott - University of Toronto - Canada

04:15 PM - 04:40 PM Fine-Tuning Cell-Autonomous Immunity: Emerging Regulators of Anti-Mycobacterial Autophagy

Michael Shiloh - University of Texas Southwestern - United States of America

BREAK - COFFEE BREAK/ INTERMISSION FOR SESSION TRANSITION

04:40 PM to 05:10 PM

KEYNOTE CONFERENCE 3 - MOBILISING GAMMA-DELTA T CELLS AGAINST CANCER

05:10 PM to 06:00 PM

PLENARY SESSION

Bruno Silva-Santos - Gulbenkian Institute for Molecular Medicine - Portugal

Chair: Herbert Leonel de Matos Guedes - Federal University of Rio de Janeiro - Brazil

POSTER SESSION 1

06:10 PM to 08:10 PM

1ST FLOOR - POOL AREA

October 15th, Wednesday

SBI-BD AWARD - SESSION I - SBI YOUNG TALENT

08:00 AM to 08:50 AM

ROOM FERRADURA & JOÃO FERNANDES

Chairs: Kenneth J. Gollob - Albert Einstein Hospital - Brazil; Claudia Ida Brodskyn - Fiocruz - Brazil; Alberto Nóbrega - Federal University of Rio de Janeiro - Brazil

08:00 AM to 08:16 AM - **Caracterização do infiltrado imune tumoral em pacientes com câncer endométrio: estratégia para a descoberta de alvos imunoterapêuticos.** Presenter: Maria Luisa Marques Pierre, - A.C. Camargo Hospital - Brazil

08:17 AM to 08:33 AM - **Desenvolvimento de um ensaio de competição multiplex por citometria de fluxo para detecção de anticorpos bloqueadores do receptor inibitório imune PD-1.** Presenter: Veridiane Maria Pscheidt - Federal University of Health Sciences of Porto Alegre - Brazil

08:34 AM to 08:50 AM - **Desvendando a importância dos compartimentos adiposos viscerais para a resposta à vacinas de mucosa.** Presenter: Guilherme William da Silva - University of São Paulo - Brazil

BD MINI COURSE - DESIGN: THE POWER OF PERFORMANCE

08:00 AM to 08:50 AM

ROOM GERIBÁ & TARTARUGA

Guilherme Evangelista - Brazil

COURSE - UNIVERSE OF EXTRACELLULAR VESICLES (EVS)

08:00 AM to 08:50 AM

ROOM TUCUNS

Topic - Protocols for the Isolation, Purification, and Characterization of EVs

Patrícia Xander Batista - Unifesp - Brazil (SP)

SYMPOSIUM 07 - MUCOSAL IMMUNOLOGY

09:00 AM to 10:40 AM

ROOM FERRADURA & JOÃO FERNANDES

Chair: Alessandra D'Almeida Filardy - Federal University of Rio de Janeiro - Brazil

09:00 AM - 09:25 AM **Living on the edge – immune regulation at the airway mucosa**

Clare Lloyd - Imperial College of London - United Kingdom

09:25 AM - 09:50 AM **Impact of dual Th2+Th17+ cells and glycocalyx shedding in difficult-to-treat asthma**

Vânia Luiza Deperon Bonato - University of São Paulo - Brazil

09:50 AM - 10:15 AM **Decoding intercellular communication that drives gut inflammation and tolerance**

Maria Cecília Canesso - Rockefeller University - United States of America

10:15 AM - 10:40 AM **Mucosal Immunity at the Crossroads: Gut Microbiota and Immune Modulation in Liver Fibrosis and Cancer**

Angelica Thomaz Vieira - University of Strasbourg - France

SYMPOSIUM 08 - IMMUNOMETABOLISM

09:00 AM to 10:40 AM

ROOM GERIBÁ & TARTARUGA

Chair: Pedro Manoel Mendes de Moraes Vieira - University of Campinas - Brazil

09:00 AM - 09:25 AM **Inflammatory metabolic rewiring as a defence strategy**

Miguel Soares - Gulbenkian Institute for Molecular Medicine - Portugal

09:25 AM - 09:50 AM **Immunometabolic control of cell death**

Peter Murray - Max Planck Institute - Germany

09:50 AM - 10:15 AM **Trained immunity vaccination in asthma**

Eva Kaufmann - Queen's University - Canada

10:15 AM - 10:40 AM **Genetically determined cytokine-induced mitochondrial dysfunction: a new paradigm and therapeutic target in the pathogenesis of Chagas disease cardiomyopathy**

Edecio Cunha Neto - University of São Paulo - Brazil

SYMPOSIUM 09 - IMMUNE RESPONSES TO PARASITES

09:00 AM to 10:40 AM

ROOM TUCUNS

Chair: Claudia Ida Brodskyn - Fiocruz - Brazil

09:00 AM - 09:25 AM **Hypoxia and CD8 T cells in cutaneous leishmaniasis**

Fernanda O. Novais - Ohio State University - United States of America

09:25 AM - 09:50 AM **A Tale of Changing Personalities: Treg Cell Heterogeneity and Its Stage-Dependent Impact in T. cruzi Infection**

Eva Virginia Acosta Rodríguez - National University of Córdoba - Argentina

09:50 AM - 10:15 AM **When T. cruzi Meets the Skin: Fibroblast and Keratinocyte Mediated NK Cell Responses During Early Infection**

Rosa Isela Gálvez - Bernhard Nocht Institute for Tropical Medicine - Germany

10:15 AM - 10:40 AM **IL-11 orchestrates pulmonary mucosal immunity during helminth infection**

Pedro Gazzinelli-Guimarães - George Washington University - United States of America

BREAK - COFFEE BREAK/ INTERMISSION FOR SESSION TRANSITION

10:40 AM to 11:10 AM

KEYNOTE CONFERENCE 4 - NECROPHAGY, COPROPHAGY, DANGEROUS INDIGESTION AND IMMUNITY TO CANCER

11:10 AM to 12:00 PM

PLENARY SESSION

Caetano Reis e Sousa - Francis Crick Institute - United Kingdom

Chair: Claudia Ida Brodskyn - Fiocruz - Brazil

SPONSORED SYMPOSIUM - DBR / CYTEK

12:00 PM to 12:30 PM

PLENARY SESSION

See the Unseen: Insights Into Autofluorescence Extraction With Cytex's High-Resolution Spectral Technology

Maria C. Jaimes, MD - United States of America

INTERMISSION FOR SESSION TRANSITION

12:30 PM to 01:00 PM

AWARD - THEREZA KIPNIS AWARD

01:00 PM to 02:50 PM

ROOM 1 - FERRADURA & JOÃO FERNANDES

Chair: Marcelo Torres Bozza - Federal University of Rio de Janeiro - Brazil

01:10 PM - 01:22 PM Dysregulated NLRP3 and NLRP1 Inflammasome Activation in Neutrophils from People Living with HIV: An MMP-9-Mediated Pathway Linking Inflammation and Endothelial Damage

Vinícius Nunes Cordeiro Leal - Brazil

01:28 PM - 01:40 PM RAB5c orchestrates LC3-associated phagocytosis to promote microbicidal function of macrophages

Edismauro Garcia Freitas Filho - Brazil

01:46 PM - 01:58 PM Microbiota-Independent Development of Natural B Cell Reactivity to a Self-Antigen and Its Bacterial Ortholog

Gustavo Meira Chaves de Assis Pereira - Brazil

02:04 PM - 02:16 PM B cell-orchestrated thermoregulation through maternal antibodies and gut microbiota

Paulo de Melo - Brazil

02:22 PM - 02:34 PM Pharmacological GSK3 inhibition prevents and reverses BBB permeability, neuroinflammation, and cognitive impairment in a mouse model of Long COVID

Remy Martins Goncalves - Brazil

MESA-REDONDA DIVULGAÇÃO CIENTÍFICA / ROUND TABLE SCIENCE COMMUNICATION

01:00 PM to 02:50 PM

ROOM GERIBÁ & TARTARUGA

Chair: Ana Maria Caetano Faria - Federal University of Minas Gerais - Brazil

01:10 PM - 01:35 PM **A Importância da Comunicação Científica na sua Carreira**

Helder Imoto Nakaya - Albert Einstein Hospital - Brazil

01:35 PM - 02:00 PM **Experiências na Ciência em Educação e Divulgação Científica da Fiocruz Bahia**

Valéria Borges - Fiocruz - Bahia - Brazil

02:00 PM - 02:25 PM **Minha trajetória como Divulgadora Científica (participação on-line)** -

Mellanie Fontes-Dutra - Unissinos - Brazil

02:25 PM - 02:50 PM **Debate**

BD FACSDISCOVER SYMPOSIUM - THE NEW ERA OF REAL-TIME IMAGING CYTOMETRY

01:30 PM to 02:30 PM

ROOM TUCUNS

Guilherme Ferreira de Brito Evangelista - Brazil

SYMPOSIUM 10 - INNATE IMMUNITY II

03:00 PM to 04:40 PM

ROOM FERRADURA & JOÃO FERNANDES

Chair: Dario S. Zamboni - University of São Paulo - Ribeirão Preto - Brazil

03:00 PM - 03:25 PM **Regulation of innate immunity**

Jonathan Kagan - Harvard Medical School - United States of America

03:25 PM - 03:50 PM **Consequences of inflammasome activation in the intestinal epithelium**

Isabella Rauch - Oregon Health & Science University - United States of America

03:50 PM - 04:15 PM **The dark side of mosquito immunity: Phagocytes promote viral dissemination**

João Trindade Marques - University of Strasbourg - France

04:15 PM - 04:40 PM **Close encounters of the DNA extracellular traps kind: The abduction of SARS-CoV-2**

Elvira M. Saraiva - Federal University of Rio de Janeiro - Brazil

SYMPOSIUM 11 - IMMUNOLOGICAL AXES

03:00 PM to 04:40 PM

ROOM GERIBÁ & TARTARUGA

Chair: Tatiani Uceli Maioli - Federal University of Minas Gerais - Brazil

03:00 PM - 03:25 PM **Behavioral outputs of gut immune responses**

Esther Borges Florsheim - Arizona State University - United States of America

03:25 PM - 03:50 PM **Dissecting allostatic physiological circuits within the oral–gut axis**

Gustavo Andrés Monasterio Ocares - Karolinska Institutet - Sweden

03:50 PM - 04:15 PM **Understanding the impact of intestinal infection in lung immunity and immune surveillance**

Denise Moraes da Fonseca - University of São Paulo - Brazil

04:15 PM - 04:27 PM **Short talk 1 - MerTk-mediated signaling boosts cigarette smoke lung inflammation mediating resistance to pneumococcal infection by modulating monocyte-derived alveolar macrophage** | Presenter: Kamila Guimarães Pinto

04:27 PM - 04:40 PM **Short talk 2 - Fiber-deprived diet weakens lung defense against antimicrobial-resistant *Klebsiella pneumoniae* and facilitates resistance phenotype in the gut microbiota** | Presenter: Mayra Fernanda Ricci

SYMPOSIUM 12 - ION CHANNELS & PURINERGIC SIGNALING IN THE IMMUNE SYSTEM

03:00 PM to 04:40 PM

ROOM TUCUNS

Chair: Robson Coutinho-Silva - Federal University of Rio de Janeiro - Brazil

03:00 PM - 03:25 PM **P2RX7 as a tissue damage sensor for pathogenic CD4+ T cell fate decisions in lung infections**

Maria Regina D'Império Lima - University of São Paulo - Brazil

03:25 PM - 03:50 PM **Fueling the potential of exhausted CD8+ T cells with extracellular ATP**

Henrique Borges da Silva - Mayo Clinic - United States of America

03:50 PM - 04:15 PM **Purinergic signaling dampens the cardiac immune response in experimental and human Chagas cardiomyopathy**

Maria Pilar Aoki - National University of Córdoba - Argentina

04:15 PM - 04:40 PM **Anion channels and transporters in T lymphocyte development and function**

Axel Concepcion - University of Chicago - United States of America

BREAK - COFFEE BREAK//INTERMISSION FOR SESSION TRANSITION

04:40 PM to 05:10 PM

KEYNOTE CONFERENCE 5 - ROLE OF KRUPPEL-LIKE FACTORS IN CD8+ T CELL TRAFFICKING AND DIFFERENTIATION

05:10 PM to 06:00 PM

PLENARY SESSION

Stephen Jameson - University of Minnesota - United States of America

Chair: Kenneth Gollob - Albert Einstein Hospital - Brazil

POSTER SESSION 2

06:00 PM to 08:00 PM

1ST FLOOR - POOL AREA

October 16th, Thursday

SBI-BD AWARD - SESSION 2 - SBI Researcher

08:00 AM to 08:50 AM

ROOM FERRADURA & JOÃO FERNANDES

Chairs: Alberto Nóbrega - Federal University of Rio de Janeiro - Brazil; Claudia Ida Brodskyn - Fiocruz - Brazil; João P. Viola - INCA - Brazil

08:00 AM to 08:16 AM - **Proposta Metodológica Multiplex com Aplicação em Vigilância Epidemiológica Ampliada.** Presenter: Laila Sampaio Horta - Fiocruz Bahia - Brazil

08:17 AM to 08:33 AM - **Eixo intestino-pulmão: reflexos da infecção intestinal sobre a imunovigilância pulmonar.** Presenter: Josiane Betim de Assis - University of São Paulo - Brazil

08:34 AM to 08:50 AM - **Análise multiparamétrica do sangue periférico e do microambiente tumoral em pacientes com câncer de cólon.** Presenter: João Figueira Scarini - Albert Einstein Hospital - Brazil

BRIGHT SPARKS 7 - INFECTIOUS DISEASES II

08:00 AM to 08:50 AM

ROOM GERIBÁ & TARTARUGA

Chair: Maria Regina D'Imperio Lima - University of São Paulo - Brazil

08:00 AM - 08:10 AM **The differential role of Gasdermin D in murine astrocytes and microglia in response to Toxoplasma gondii**

Amanda Verdicchio Caniato - Brazil

08:12 AM - 08:22 AM **Early immune signatures predict disease tolerance and resistance in COVID-19 progression**

Elena Victoria Montes Cobos - Spain

08:24 AM - 08:34 AM **The deleterious role of Gasdermin-D (GSDMD) in the control of Toxoplasma gondii by macrophages**

Rafael Queiroz de Souza - Brazil

08:36 AM - 08:46 AM **Longitudinal immune response to a new member of the Plasmodium vivax erythrocyte binding protein family (EBP2) in individuals from the Brazilian Amazon**

Gabriela Maia Fernandes - Brazil

BECKMAN COULTER LIFE SCIENCES - MINI COURSE: SMALL PARTICLES & FLOW CYTOMETRY: SIZE MATTERS, BUT IT'S COMPLICATED!

08:00 AM to 08:50 AM

ROOM TUCUNS

Speaker: Vera Tang - Flow Cytometry Manager - University of Ottawa - Canada

Moderators: Ana Claudia Trocoli Torrecilhas - UNIFESP - Brazil; Patrícia Xander Batista - UNIFESP - Brazil; Raquel Ferraz-Nogueira - Brazil

SYMPOSIUM 13 - INFECTIOUS TROPICAL DISEASES

09:00 AM to 10:40 AM

ROOM FERRADURA & JOÃO FERNANDES

Chair: Walderez Dutra - Federal University of Minas Gerais - Brazil

09:00 AM - 09:25 AM **Severe malaria: Implications for a regulatory network of CD8⁺ T cells and Tr1 cells**

Thomas Jacobs - Bernhard Nocht Institute for Tropical Medicine - Germany

09:25 AM - 09:50 AM **Single-cell transcriptomics reveals subset-specific immune signatures in indeterminate and cardiac Chagas disease patients**

Walderez Dutra - Federal University of Minas Gerais - Brazil

09:50 AM - 10:15 AM **Pathogen-free gut microbiota enhances malaria severity inducing intestinal and systemic tolerogenic immune response**

Rafael Polidoro Alves Barbosa - Indiana University School of Medicine - United States of America

10:15 AM - 10:27 AM **Short talk 1: Spatial profiling of human diffuse cutaneous leishmaniasis lesions reveals immunosuppressive microenvironments that coincide with parasite localisation** | Presenter: Carlos Henrique Dettmann Fantecelle de Castro

10:28 AM - 10:40 AM **Short talk 2: Innate and adaptive immune signatures distinguish clinical presentations in a malaria cohort revealed by high-dimensional flow cytometry** | Presenter: Laila Horta

SYMPOSIUM 14 - T CELL RESPONSES

09:00 AM to 10:40 AM

ROOM GERIBÁ & TARTARUGA

Chair: Gustavo Amarante-Mendes - University of São Paulo - Brazil

09:00 AM - 09:30 AM **How early-life programming of intestinal T lymphocytes shapes tolerance**

Angelina Bilate - Rockefeller University - United States of America

09:30 AM - 10:00 AM **Control of regulatory T cell differentiation and function**

José Carlos Alves-Filho - University of São Paulo - Ribeirão Preto - Brazil

10:00 AM - 10:30 AM **Control of CD8 T cell function by Polycomb Group proteins**

Renata M. Pereira - Federal University of Rio de Janeiro - Brazil

SYMPOSIUM 15 - IMMUNITY & CELL COMMUNICATION

09:00 AM to 10:40 AM

ROOM TUCUNS

Chair: Elvira M. Saraiva - Federal University of Rio de Janeiro - Brazil

09:00 AM - 09:25 AM From guardians to neighbors: Macrophage diversity in intercellular communication across tissues

Fernando Martinez Estrada - University of Surrey - United Kingdom

09:25 AM - 09:50 AM The immune facets of megakaryocytes in viral chronicity

Fernando Real - Institut Pasteur de Lille - France

09:50 AM - 10:15 AM Neutrophil-derived extracellular vesicles in host-parasite interaction during Leishmania infection

Anderson Guimarães Costa - Federal University of Rio de Janeiro - Brazil

10:15 AM - 10:27 AM Short talk 1: Neutrophil extracellular vesicles as mediators of tumor plasticity: a new perspective in cancer immunotherapy | Presenter: Carolinne Souza de Amorim

10:28 AM - 10:40 AM Short talk 2: Adipose tissue-derived small extracellular vesicles as key mediators of the immune response and tumor microenvironment modulation | Presenter: Letícia Aparecida Ferreira Venerando

COFFEE BREAK/ INTERMISSION FOR SESSION TRANSITION

10:40 AM to 11:10 AM

KEYNOTE CONFERENCE 6 - INTER-ORGAN DIVERSITY OF TISSUE-RESIDENT LYMPHOCYTES

11:10 AM to 12:00 PM

PLENARY SESSION

Laura Mackay - The University of Melbourne - Australia

Chair: Ana Maria Caetano Faria - Federal University of Minas Gerais - Brazil

BECKMAN COULTER LIFE SCIENCES - SMALL PARTICLES & FLOW CYTOMETRY: APPLICATIONS IN IMMUNOLOGY AND VIROLOGY

12:00 PM to 12:50 PM

PLENARY SESSION

Vera Tang - Flow Cytometry Manager - University of Ottawa - Canada

COFFEE BREAK/ INTERMISSION FOR SESSION TRANSITION

12:50 PM to 01:20 PM

GENERAL ASSEMBLY SBI

01:20 PM to 03:00 PM

ROOM FERRADURA & JOÃO FERNANDES

BRIGHT SPARKS 8 - INNATE IMMUNITY

01:30 PM to 03:00 PM

ROOM GERIBÁ & TARTARUGA

Chair: Rafael Polidoro - Indiana University School of Medicine - United States of America

01:30 PM - 01:40 PM **MicroRNAs-205 and -193b modulate the response of human keratinocytes and macrophages to Leishmania braziliensis infection**

Sara Nunes de Oliveira Araujo - Brazil

01:42 PM - 01:52 PM **Trained immunity induced by Bacillus Calmette-Guérin (BCG) enhances systemic antitumor response improving bladder cancer treatment**

Nina Mari Gual Pimenta de Queiroz - Brazil

01:54 PM - 02:04 PM **α -Galactosidase A deficiency primes and potentiates inflammasome activation in a cellular model of Fabry disease**

Ana Beatriz Figueiredo de Lima - Brazil

02:06 PM - 02:16 PM **Mycobacterial-induced lipid droplets function as an immunoinflammatory platform in DCs through DGAT-1 and PGE2, contributing to M. bovis BCG burden**

Guilherme lack Pinheiro da Cruz - Brazil

02:18 PM - 02:28 PM **Impaired CD1c⁺ dendritic cell responses and lipid metabolism dysregulation in severe COVID-19**

Geovane Marques Ferreira - Brazil

02:30 PM - 02:40 PM **Inhibition of tonic PD-1 signaling in dendritic cells enhances costimulatory pathways**

Rodrigo Benedetti Gassen - Brazil

02:42 PM - 02:52 PM **Epigenetic modulation as a promising approach to enhance NK cell functional features in breast cancer patients**

Daniela Delechiave - Brazil

BRIGHT SPARKS 9 - IMMUNOTHERAPY

01:30 PM to 03:00 PM

ROOM TUCUNS

Chair: Henrique Borges da Silva - Mayo Clinic - United States of America

01:30 PM - 01:40 PM **Evaluation of the effect of the co-culture with dendritic cells in the phenotype and function of iPSC-derived CAR-T cells**

Luana Correia Croda Catharino - Brazil

01:42 PM - 01:52 PM **Human CAR-engineered macrophages from cord blood hematopoietic stem cell to treat HER2+ solid tumors**

Ian Costa Ovider - Brazil

01:54 PM - 02:04 PM **CBP/p300 inhibition enhances stem-like phenotype while limiting regulatory polarization in CAR-T cells against hematologic and solid tumors**

Maria Leticia Rodrigues Carvalho - Brazil

02:06 PM - 02:16 PM **Generation of CD19 CAR-T cells using a minicircle-Sleeping Beauty transposon platform**

Leonardo Ribeiro Batista Silva - Brazil

02:18 PM - 02:28 PM **Creation of a platform to generate dendritic cells expressing chimeric antigen receptors from human induced pluripotent stem cell to treat tumors**

Paula do Amaral Costa Ribeiro - Brazil

02:30 PM - 02:40 PM **Modified extracellular vesicles for STAT3 inhibition and activation of innate immune responses for cancer treatment**

Jordy Alexander Larco Lasso - Ecuador

02:42 PM - 02:52 PM **GITRL coexpression enhances CAR-T cell resistance to exhaustion and sustains antitumor activity in a glioblastoma model**

Izadora Peter Furtado - Brazil

SYMPOSIUM 16 - ONTOGENY & IMMUNITY AT DIFFERENT LIFE STAGES

03:00 PM to 04:40 PM

ROOM FERRADURA & JOÃO FERNANDES

Chair: Ana Maria Caetano Faria - Federal University of Minas Gerais - Brazil

03:00 PM - 03:25 PM **Embryonic lymphoid cells shape thymic function for life**

Ana Cumano - Institut Pasteur - France

03:25 PM - 03:50 PM **“Born to Be Wired”: How the gut microbiota programs the newborn hepatic immune system**

Gustavo Menezes - Federal University of Minas Gerais - Brazil

03:50 PM - 04:15 PM **Inflammation induces immunity to become auto-aggressive during ageing**

Arne Akbar - University College of London - United Kingdom

04:15 PM - 04:27 PM **Short talk 1: NFATc1 transcription factor is required to maintain a fetal-exclusive human B-cell progenitor** | Presenter: Olivia Fonseca Souza

04:28 PM - 04:40 PM **Short talk 2: Metabolites increased after the reconstitution of milk microbiota are able to modulate the neonatal intestinal immunity in a colonization-dependent manner** | Presenter: Lucas Soveral

SYMPOSIUM 17 - B CELLS & ANTIBODIES II

03:00 PM to 04:40 PM

ROOM GERIBÁ & TARTARUGA

Chair: André Macedo Vale - Federal University of Rio de Janeiro - Brazil

03:00 PM - 03:25 PM **Tolling and ChATting of B cells – regulation and functions of B cells in infection**

Nicole Baumgarth - Johns Hopkins Bloomberg School of Public Health - United States of America

03:25 PM - 03:50 PM **Hardwired specificity: natural anti-HSC70 antibodies emerge independently of microbial orthologs**

André Macedo Vale - Federal University of Rio de Janeiro - Brazil

03:50 PM - 04:15 PM **B cells in metabolic disorder, antibody diversification, and beyond**

Thomas Hägglöf - Einstein Medical School - United States of America

04:15 PM - 04:27 PM **Short talk 1: Naturally occurring neutralizing anti-checkpoint antibodies predict survival in advanced lung cancer patients** | Presenter: Fernanda Tereza Bovi Frozza

04:28 PM - 04:40 PM **Short talk 2: Rapamycin selectively modulates B-1 lymphocyte function and points to feedback regulation of the PI3K/mTOR pathway** | Presenter: Caio Omori Del Debbio Pinheiro

SYMPOSIUM 18 - INFECTION-DRIVEN LONG-TERM IMMUNE DISORDERS

03:00 PM to 04:40 PM

ROOM TUCUNS

Chair: Patricia Torres Bozza - FIOCRUZ - Rio de Janeiro - Brazil

03:00 PM - 03:25 PM **Cerebrovascular Wnt/beta-catenin and Caveolin-1 regulate Long COVID neuroinflammation and cognitive impairment**

Sarah E. Lutz - University of Illinois - United States of America

03:25 PM - 03:50 PM **Unraveling the immunopathology of infection-associated chronic illnesses: sex differences and predictive diagnostics**

Michal (Mikki) Caspi Tal - Massachusetts Institute of Technology - United States of America

03:50 PM - 04:15 PM **After the virus: what comes next?**

Vivian Vasconcelos Costa - Federal University of Minas Gerais - Brazil

04:15 PM - 04:27 PM **Short talk 1: Disruption of pulmonary immune surveillance following intestinal infection** | Presenter: Josiane Betim de Assis

04:28 PM - 04:40 PM **Short talk 2: Microbiota-driven inflammatory imprints and gut–lung–brain axis activation in a murine model of Long COVID-like pathology** | Presenter: Jackeline Marino Lucas

COFFEE BREAK/ INTERMISSION FOR SESSION TRANSITION

04:40 PM to 05:10 PM

KEYNOTE CONFERENCE 7 - DISCRIMINATION OF SELF AND NON-SELF RNA BY THE INNATE IMMUNE SYSTEM

05:10 PM to 06:00 PM

PLENARY SESSION

Veit Hornung - Ludwig-Maximilians-Universität - Germany

Chair: Dario S. Zamboni - University of São Paulo - Ribeirão Preto - Brazil

POSTER SESSION 3

06:00 PM to 08:00 PM

1ST FLOOR - POOL AREA

October 17th, Friday

BRIGHT SPARKS 10 - TUMOR IMMUNOLOGY

08:00 AM to 09:40 AM

ROOM FERRADURA

Chair: Letícia Carneiro - Federal University of Rio de Janeiro - Brazil

08:00 AM - 08:10 AM **Circulating monocytes from newly diagnosed multiple myeloma patients display a pro-inflammatory gene signature**

Viviane Jennifer da Silva - Brazil

08:12 AM - 08:22 AM **Linking baseline immune mediators and tumor microenvironment features to disease recurrence in colon cancer**

João Figueira Scarini - Brazil

08:24 AM - 08:34 AM **Multi-omic metabolic signatures for tumor immunity classification and prognosis**

Higor Almeida Cordeiro Nogueira - Brazil

08:36 AM - 08:46 AM **Positive regulatory loop of Galanin/Galanin Receptor 2 (GALR2) and CCL2/CCR2 axes in head and neck squamous cell carcinoma (HNSCC)**

Fourogh Alemiserej - Iran

08:48 AM - 08:58 AM **Tumor compartment-specific shifts in macrophage phenotypes underlie distinct immune interactions in colorectal cancer**

Igor Salerno Filgueiras - Brazil

09:00 AM - 09:10 AM **Epithelial proliferation triggered by inulin is mediated by $\gamma\delta$ T cells through microbiota remodeling and linked to anti-tumor activity**

Helder Carvalho de Assis - Brazil (MG)

09:12 AM - 09:22 AM **Epigenetic blockade of CBP/p300 reinvigorates exhausted TIL and boosts immune checkpoint blockade in metastatic melanoma**

Bruno Dalbello da Silva Elias - Brazil

09:24 AM - 09:34 AM **Engineering NK cells to optimise liver homing and block PD-L1-mediated inhibition of T cells for adoptive cell therapy of liver cancer**

Mariana de Oliveira Diniz - Brazil

BRIGHT SPARKS 11 - IMMUNOPHARMACOLOGY & IMMUNOTHERAPY

08:00 AM to 09:40 AM

ROOM JOÃO FERNANDES

Chair: Claudia Benjamim - Federal University of Rio de Janeiro - Brazil

08:00 AM - 08:10 AM **Targeting leukocyte recruitment: optimized CXCL9-derived peptides for inflammation control**

Vívia Louise Soares de Oliveira - Brazil (MG)

08:12 AM - 08:22 AM **A formulation based on an Annexin A1-derived peptide: an anti-inflammatory and pro-resolving therapy against severe dengue**

Jenniffer Ramos Martins - Brazil

08:24 AM - 08:34 AM **Annexin A1 as a therapeutic target in severe malaria: implications for neuroprotection and pulmonary function**

Natália Melo - Brazil (MG)

08:36 AM - 08:46 AM **Activation of NLRP3 inflammasome by Bothropstoxin-I, from B. jararacussu snake venom, and its impact on muscle regeneration of AIRmax and AIRmin mice**

Isabella Mitie de Camargo - Brazil

08:48 AM - 08:58 AM **Effect of Aedes aegypti salivary components on experimental sepsis: neutrophil modulation**

Júlia de Moura Bernardi - Brazil

09:00 AM - 09:10 AM **A bead-based assay for the screening of monoclonal antibodies that disrupt PD 1/PD-L1 interaction**

Veridiane Maria Pscheidt - Brazil

09:12 AM - 09:22 AM **Validating a manufacturing process for CAR-T cells accessible to the Brazilian healthcare system for treating pediatric hematologic cancers**

Sabrina Alves dos Reis - Brazil

09:24 AM - 09:34 AM **Bioimaging Brasil: democratizing intravital microscopy across Brazil**

Maísa Mota Antunes - Brazil (MG)

BRIGHT SPARKS 12 - IMMUNOMETABOLISM

08:00 AM to 09:40 AM

ROOM GERIBÁ

Chair: Heitor de Paula Neto - Federal University of Rio de Janeiro - Brazil

08:00 AM - 08:10 AM **The CCL17–CCL22/CCR4 chemokine axis safeguards against obesity-induced metabolic imbalance**

Vanderlei da Silva Fraga Junior - Brazil

08:12 AM - 08:22 AM **Rho-kinase as a central regulator of hepatic insulin resistance, senescence, and inflammation during aging**

Vitor Rosetto Muñoz - Brazil

08:24 AM - 08:34 AM **High glucose heightens vulnerability to Leishmania braziliensis infection in human macrophages by hampering the production of reactive oxygen species through TLR2 and TLR4**

Rana Pereira dos Santos Bastos - Brazil

08:36 AM - 08:46 AM **IL-21 induces neuroinflammation and alters lipid metabolism**

Hugo Akirito de Almeida Oyamada - Brazil

08:48 AM - 08:58 AM **Maternal high-fat diet alters immune lung landscape in neonatal mice via ST2/IL-33 axis modulation**

Laura Machado Menegati - Brazil (MG)

09:00 AM - 09:10 AM **Contribution of microbiota and regulatory T cells for the protection against metabolic syndrome conferred by mucosal infection**

Bernardo de Castro Oliveira - Brazil

09:12 AM - 09:22 AM **Inflammasome inhibition by modulation of lipid metabolism during SARS-CoV-2 infection**

Vinicius Cardoso Soares - Brazil (RJ)

09:24 AM - 09:34 AM **Evaluation of NAD metabolism during monocyte maturation into macrophages and its influence on cell activation by neutrophil extracellular traps (NETs)**

Marcos A. Formiga Junior - Brazil

BRIGHT SPARKS 13 - INFLAMMATION

08:00 AM to 09:40 AM

ROOM TARTARUGA

Chair: Fabianno Dutra - Federal University of Rio de Janeiro - Brazil

08:00 AM - 08:10 AM **Amoxicillin-induced gut dysbiosis triggers a pro-inflammatory state that impairs reproduction – a possible gut–reproduction axis**

Evandro Neves Silva - Brazil (MG)

08:12 AM - 08:22 AM **PPAR γ -mediated effects of oleic acid on macrophages highlight a potential therapeutic target in psoriasis**

Beatriz Burger - Brazil (SP)

08:24 AM - 08:34 AM **Extracellular vesicles carrying tissue factor promote neutrophil aggregation during Long COVID**

Thiago Soares de Souza Vieira - Brazil

08:36 AM - 08:46 AM **The adenosinergic signaling regulates the severity of sepsis and the neutrophil extracellular traps release**

Ana Flávia Oliveira Pampolha - Brazil

08:48 AM - 08:58 AM **Disease tolerant mice shelter a pathogenic intestinal microbiota able to trigger lethal disease in low tolerant hosts**

Vinicius Mendes Vidal - Brazil

09:00 AM - 09:10 AM **DNA extracellular traps released by Mayaro virus-infected macrophages act as a platform for virus dissemination**

Ana Salles de Carvalho - Brazil

09:12 AM - 09:22 AM **Transcriptional repression of Tnfa and Il12 by T-bet in macrophages attenuates obesity-driven inflammation and metabolic dysregulation**

Webster Leonardo Guimarães da Costa - Brazil

09:24 AM - 09:34 AM **Rare genetic variants of NLRP12 in admixed Latino-American children with SARS-CoV-2-related multisystem inflammatory syndrome**

Raquel Bispo de São Pedro - Brazil

BRIGHT SPARKS 14 - VACCINES

08:00 AM to 09:40 AM

ROOM TUCUNS

Chair: Silvia Beatriz Boscardin - USP - Brazil (SP)

08:00 AM - 08:10 AM **Immunization with Trypanosoma cruzi trans-sialidase RNA sequences or recombinant proteins containing SAPA repeats protects mice against infection and promotes a balanced inflammatory response**

Nailma Silva Aprigio dos Santos - Brazil

08:12 AM - 08:22 AM **Distinct adjuvant strategies and antigen multimerization potentiate immunity induced by a SARS-CoV-2 RBD subunit vaccine**

João Pedro da Silva Nunes - Brazil

08:24 AM - 08:34 AM **Identification of rare genetic variants in individuals with severe neurological adverse events following the administration of ChAdOx1 nCoV-19 vaccine in Brazil**

Milena Regina Gomes Lopes - Brazil

08:36 AM - 08:46 AM **Uncovering immune profiles in yellow fever patients with prior 17DD vaccination: insights from the 2016-2018 Brazilian outbreak**

Mariane Juliele Mendes - Brazil

08:48 AM - 08:58 AM **The impact of orthopoxvirus vaccination and Mpox infection on cross-protective immunity**

Jameson Crandell - United States of America

09:00 AM - 09:10 AM **Exploring a NS1-based mRNA vaccine for dengue: antibody and T cell responses**

Sarah A. R. Sérgio - Brazil

09:12 AM - 09:022 AM **GLP production and characterization of the L3 ribosomal protein for a universal malaria vaccine (UniMaV)**

Luna Barroco de Lacerda - Brazil

BREAK/ INTERMISSION FOR SESSION TRANSITION

09:40 AM to 10:10 AM

ROUNDTABLE - GRADUATE COURSES IN IMMUNOLOGY IN BRAZIL / MESA REDONDA: DEBATE SOBRE A PÓS-GRADUAÇÃO NO BR (EM PORTUGUÊS)

10:10 AM to 11:30 AM

PLENARY SESSION

Chair: Alessandra D'Almeida Filardy - Federal University of Rio de Janeiro - Brazil

10:00 AM - 10:40 AM **O panorama da pós graduação em Imunologia no Brasil**

Camila Indiani de Oliveira - Fiocruz - Bahia - Brazil

10:40 AM - 11:10 AM **Visão da área da Ciência Biológica II sobre a pós graduação**

Débora Foguel - Federal University of Rio de Janeiro - Brazil

11:10 AM - 11:30 AM **Debate**

KEYNOTE CONFERENCE 8 - CLOSING CONFERENCE - IMPACT OF THE ENVIRONMENT ON IMMUNOSENESCENCE

11:30 AM to 12:30 PM

PLENARY SESSION

Ana Maria Caetano Faria - Federal University of Minas Gerais - Brazil

Chair: Momtchilo Russo - USP - Brazil (SP)

AWARD - AWARDS ANNOUNCEMENTS AND CLOSING REMARKS

12:30 PM to 01:30 PM

PLENARY SESSION

SPONSORED BY

DIAMOND



GOLD



SILVER



BRONZE



SUPPORT



Dear Friends and Colleagues,

On behalf of the Brazilian Society of Immunology (SBI), we are pleased and honored to invite you to attend our XLIX Annual Congress, which will take place in the town of Armação dos Búzios, from October 13th to 17th, 2025.

The Scientific Program is composed of symposia, round tables and keynote lectures on recent advances in various fields of Immunology, including: lymphocyte development and function, immune responses to pathogens, mucosal immunology, immunometabolism, tumor immunology, autoimmune and inflammatory disorders, immunosenescence, and much more. We are delighted to host several worldwide leaders and young investigators in Immunology and related areas, who will present their most recent data and personal views. Additionally, an introductory Immunology course on Regulatory T cells and a Satellite Meeting on Vaccines will be held on October 12th and 13th, just before the main SBI Congress. A course on Extracellular Vesicles will also complement the scientific program.

Following SBI tradition, we will give special attention to undergraduate and graduate students, as well as post-doctoral fellows, who will present their ongoing work, selected from submitted abstracts, during 8 Bright Spark and 3 Poster Sections.

As every other year, the SBI Lifetime Achievements in Immunology Award will be granted to an SBI member, nominated by an independent committee, in recognition of their scientific achievements in Immunology, commitment to mentoring, contributions to the development of Science in Brazil and service to SBI. Both the now traditional Thereza Kipnis Award and the SBI-BD Cytometry Award will also be given: don't miss the special sections when the finalist candidates present their works.

This year, we will also have some novelties: a round table discussing Graduate Courses in Immunology in Brazil and another debating Science Communication.

Participants will have the chance to enjoy the scientifically rich yet relaxed atmosphere to learn, exchange experiences and build collaboration networks. The meeting location, Armação dos Búzios, often referred to simply as Búzios, will be an additional attraction. Búzios is a charming and stylish town located at the coast of Rio de Janeiro State, featuring 23 beautiful beaches along an 8-kilometer-long peninsula. It gained international attention and became one of the most sought-after tourist destinations after the visit of Brigitte Bardot, the famous French actress, in the 1960s. Despite that, Búzios continues to offer tranquility, direct contact with nature and breathtaking views. We believe it will be the perfect environment to foster scientific discussion and partnership, the main purposes of the congress.

We look forward to seeing you at our meeting!

The SBI Board of Directors

• Thereza Kipnis Award	36
• Poster Presentations	
○ Cellular Immunology (CE)	38
○ Chemokines and Trafficking (CT)	91
○ Clinical Immunology (CL)	93
○ Education in Immunology (EI)	108
○ Humoral Immunology (HI)	110
○ Immunology of Infectious and Parasitic Diseases (ID)	118
○ Immunopharmacology (IP)	196
○ Immunoregulation (IR)	216
○ Innate Immunity (IN)	239
○ Molecular Immunology (MI)	274
○ Neuroimmunology (NI)	292
○ Transplantation and Immunogenetics (TR)	309
○ Tumor Immunology (TU)	311
○ Vaccines (VC)	357
• Oral Presentations	
○ Cellular Immunology (CE)	386
○ Chemokines and Trafficking (CT)	391
○ Clinical Immunology (CL)	391
○ Education in Immunology (EI)	393
○ Humoral Immunology (HI)	393
○ Immunology of Infectious and Parasitic Diseases (ID)	394
○ Immunopharmacology (IP)	404
○ Immunoregulation (IR)	406
○ Innate Immunity (IN)	411
○ Molecular Immunology (MI)	415
○ Neuroimmunology (NI)	416
○ Transplantation and Immunogenetics (TR)	419
○ Tumor Immunology (TU)	420
○ Vaccines (VC)	432

3178 – Category: HUMORAL IMMUNOLOGY (HI)

Microbiota-Independent Development of Natural B Cell Reactivity to a Self-Antigen and Its Bacterial Ortholog

PEREIRA, G.M.C.D.A.¹; MAIA, L.C.R.¹; FERREIRA, C.L.²; HOBBS, A.²; VICTORA, G.²; NÓBREGA, A.¹; VALE, A.M.¹.
 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. ROCKEFELLER UNIVERSITY, NEW YORK - ESTADOS UNIDOS DA AMERICA.

The humoral portion of the adaptive immune response is mediated by B cells through the production of immunoglobulins. These proteins arise from somatic rearrangements of variable gene segments. Due to the enormous combinatorial diversity of these segments, some rearrangements may lead to the recognition of self-antigens. Contrary to models in which self-reactive B cells are purged during ontogeny, we detect broad autoreactivity in serum and resting subsets. The strongest targets the chaperone HSC70 in peritoneal B-1a cells. Because HSC70 is homologous to the bacterial ortholog DnaK, we asked whether recognition of one antigen shapes the response to the other. We used flow cytometry, B cell culture, limiting-dilution assays and immunoglobulin gene sequencing to analyze BALB/c and B6 mice at different developmental stages and colonization conditions. Tissues analysed included peritoneal cavity (PerC), spleen (Sp), mesenteric lymph nodes (mLN) and Peyer's patches (PP). HSC70-specific cells were highly enriched in PerC B-1a, whereas DnaK reactivity was widespread. Cross-reactive clones were exceedingly rare. Both specificities were detected in neonatal PerC and Sp irrespective of microbiota, indicating a germline origin. HSC70 binding was largely restrictive, while a fraction of the DnaK-reactive cells was polyreactive. In germ-free (GF) mice, DnaK-reactive cells peaked transiently in PPs after weaning (day 28), likely due to an acute response against microbial antigens in sterile food. This response was absent in minimally colonized mice. Cross-reactive clones appeared only in pre-germinal-center subsets, suggesting their deletion upon activation. Sequencing confirmed early anti-HSC70 clones and revealed age-related repertoire diversification and distinctive features in adult GF mice. These findings provide new insights into the origins of natural autoreactivity and challenge the assumed dependence on microbial antigens for shaping early B cell repertoires. Keywords: B lymphocytes;Intestinal Microbiota;Natural Antibodies.

3269 – Category: HUMORAL IMMUNOLOGY (HI)

B cell-orchestrated thermoregulation through maternal antibodies and gut microbiota

DE MELO, P.¹; ALVES, J.M.¹; DA SILVA, A.B.C.¹; IVALDO FILHO, .¹; SANTOS, J.D.¹; MORGAN, H.¹; ASSIS, A.P.¹; DOS SANTOS, C.V.¹; REGEN, T.²; DE OLIVEIRA, F.A.³; DOS SANTOS, J.R.⁴; RAMOS, A.D.S.¹; SARTORI, D.C.¹; NAVEGANTES, L.C.¹; CUNHA, F.Q.¹; ALVES-FILHO, J.C.¹; PASSAGLIA, R.D.C.A.T.¹; KETTELHUT, I.D.C.¹; CORREA, F.M.D.A.¹; ALBERICI, L.C.⁴; WAISMAN, A.⁵; CUNHA, T.M.¹; FERREIRA, J.C.B.⁶; ELIAS, L.L.K.¹; VINOLO, M.A.R.⁷; LEIRIA, L.O.S.¹. 1. FMRP, RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSITY MEDICAL CENTER OF THE JOHANNES GUTENBERG UNIVERSITY MAINZ, RIBEIRÃO PRETO - SP - BRASIL; 3. FMRP, RIBEIRÃO PRETO - SP - BRASIL; 4. FCFRP, RIBEIRÃO PRETO - SP - BRASIL; 5. UNIVERSITY MEDICAL CENTER OF THE JOHANNES GUTENBERG UNIVERSITY MAINZ, MAINZ - ALEMANHA; 6. ICB USP, SÃO PAULO - SP - BRASIL; 7. UNICAMP, CAMPINAS - SP - BRASIL.

Homeothermy requires coordinated neuroendocrine, behavioral, vascular and metabolic responses, yet immune components within this thermoregulatory framework remain poorly understood. We explored B cells' thermoregulatory function after identifying abundant B cell populations in BAT. Despite hyperphagia, B cell-deficient ($\mu\text{MT}^{-/-}$) mice exhibit elevated energy expenditure and enhanced glucose homeostasis but display profoundly impaired thermoregulatory capacity with lower core body temperature and cold intolerance. BAT evaluation reveals 50% mass reduction with increased noradrenaline and intact mitochondrial function, confirming normal heat production despite chronic hypothermia. Tail infrared thermography revealed greater heat loss and impaired vasomotor control, with mesenteric arteries failing sympathetic stimulation and inability to mount adaptive pressor response to cold. Heterozygous crosses showed $\mu\text{MT}^{-/-}$ littermates exhibited normal cold tolerance, prompting maternal inheritance exploration. Cross-generational cohousing enhanced cold tolerance in $\mu\text{MT}^{-/-}$ mice through bacterial transfer, with Bacteroidota restoration and direct Bacteroidaceae transfer from WT donors. Reciprocal fecal microbiota transplantation confirmed $\mu\text{MT}^{-/-}$ mice receiving WT microbiota acquired cold tolerance, reinforcing gut microbiota as pivotal homeothermic regulator. IgM α males lacking secreted antibodies exhibited cold intolerance, implicating antibody-specific mechanisms. Dual-breeding isolated maternal antibody (matAb) effects: offspring without matAb showed significantly lower CBT and thermoregulatory deficits, while rectal temperature/ infrared imaging confirmed matAb deficiency induces cold intolerance. This study provides first experimental evidence that vertically transferred immune factors establish homeothermic set points through gut microbiota colonization, revealing an essential pathway integrating immunology, microbiology, and thermal physiology. Keywords: Thermoregulation;B cells;Gut microbiota.

2767 – Category: INNATE IMMUNITY (IN)

Dysregulated NLRP3 and NLRP1 Inflammasome Activation in Neutrophils from People Living with HIV: An MMP-9-Mediated Pathway Linking Inflammation and Endothelial Damage

LEAL, V.N.C.¹; ANDRADE, M.M.D.S.²; CANTONI, J.¹; SATO, M.N.²; PONTILLO, A.¹. 1. LABORATÓRIO DE IMUNOGENÉTICA, DEPARTAMENTO DE IMUNOLOGIA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS/ICB, UNIVERSIDADE DE SÃO PAULO/USP, SÃO PAULO - SP - BRASIL; 2. LABORATÓRIO DE INVESTIGAÇÃO MÉDICA EM DERMATOLOGIA E IMUNODEFICIÊNCIAS (LIM-56), DEPARTAMENTO DE DERMATOLOGIA, HOSPITAL DAS CLÍNICAS E FACULDADE DE MEDICINA/HCFMUSP, SÃO PAULO - SP - BRASIL.

Inflammasomes such as NLRP3 and NLRP1 are multiprotein platforms that mediate IL-1 β release and inflammatory cell death. While extensively studied in macrophages and lymphocytes, their activation and function in neutrophils—particularly in people living with HIV (PLWH), a population marked by chronic inflammation—remain poorly understood. Methods: We analyzed neutrophils from 400 PLWH and 50 healthy donors. Inflammasome activation was assessed using immunofluorescence, flow cytometry, Western blotting, and functional assays including NET formation, IL-1 β release, degranulation, and MMP-9 activity. In vitro models with HL-60 cells and co-cultures with endothelial HUVECs were used to evaluate endothelial injury. We also genotyped 10 SNVs in inflammasome-related genes and correlated them with cellular and clinical findings. Results: Neutrophils from PLWH exhibited spontaneous and exacerbated activation, with increased cell death, NET release, and tertiary granule degranulation—especially of MMP-9. We identified for the first time a functionally active NLRP1 inflammasome in human neutrophils, alongside aberrant NLRP3 activation, both contributing to IL-1 β production and ASC speck formation. Importantly, we validated a novel MMP-9-dependent axis of IL-1 β processing via NLRP3, previously proposed by our group, and now confirmed in neutrophils from PLWH. Gain-of-function SNVs in NLRP1 and NLRP3 were associated with increased neutrophil activation and cardiovascular risk. In co-cultures, TNF- α -primed endothelial cells amplified neutrophil death and NET formation in an NLRP3- and MMP-9-dependent loop, which also triggered inflammasome activation in endothelial cells themselves. Conclusion: We reveal a **NLRP3-MMP-9-dependent IL-1 β pathway** and are the first to demonstrate **functional NLRP1 inflammasome activation in human neutrophils**, linking neutrophil dysfunction, genetic variants, and cardiovascular risk in PLWH. Keywords: Neutrophils; Inflammasome; PLWH.

2808 – Category: INNATE IMMUNITY (IN)

RAB5c orchestrates LC3-associated phagocytosis to promote microbicidal function of macrophages

FREITAS FILHO, E.G.¹; MOREIRA, I.Z.¹; ROCHA, M.F.¹; TERREL, D.L.A.¹; ALVES, C.B.D.A.¹; SILVA, P.A.D.C.²; PIRAINÉ, R.E.A.³; BOADA-ROMERO, E.⁴; WILEMAN, T.⁵; ALMEIDA, F.³; GOLDMAN, G.H.²; FLOREY, O.⁶; CUNHA, L.D.¹. 1. DEPARTAMENTO DE BIOLOGIA CELULAR E MOLECULAR E BIOAGENTES PATOGENICOS, FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO, UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY, RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 4. DEPARTMENT OF IMMUNOLOGY, ST. JUDE CHILDREN'S RESEARCH HOSPITAL, MEMPHIS - ESTADOS UNIDOS DA AMERICA; 5. NORWICH MEDICAL SCHOOL, UNIVERSITY OF EAST ANGLIA, NORWICH, NORFOLK - REINO UNIDO; 6. SIGNALLING PROGRAMME, BABRAHAM INSTITUTE, CAMBRIDGE - REINO UNIDO.

Non-canonical conjugation of ATG8 proteins, including LC3, to single membranes implicates the autophagy machinery in cell functions unrelated to metabolic stress. One such pathway is LC3-associated phagocytosis (LAP), which aids in phagosome maturation and subsequent signaling upon cargo uptake mediated by certain innate immunity-associated receptors. Thus, understanding LAP molecular events is key to determine the phagosome-autonomous effects on innate immune responses. Here, we show that a specific isoform of RAB5 GTPases, the molecular switches controlling early endosome traffic, is necessary for LAP. Using real-time confocal microscopy (IF), immunoblotting (IB), and biochemical assays we demonstrate that RAB5c regulates phagosome recruitment and function of complexes required for phosphatidylinositol-3-phosphate [PI(3)P] and intraphagosomally reactive oxygen species (ROS) generation by macrophages. IF and IB also showed that RAB5c facilitates phagosome translocation of the V-ATPase transmembrane core, which is needed for ATG16L1 binding and consequent LC3 conjugation and phagosome maturation. RAB5c depletion impaired macrophage elimination of the fungal pathogen *Aspergillus fumigatus*, as assessed by CFU counts. Confirming the importance of RAB5c-V-ATPase-ATG16L1 axis for macrophage killing capacity, mutant mice lacking the C-terminal domain of ATG16L1 that binds to V-ATPase (*Atg16l1^{DWD40}*) are more susceptible to *A. fumigatus* *in vivo*. Thus, early endosome-to-phagosome trafficking can be selectively engaged to promote macrophage pathogen elimination by directing phagosomal maturation toward LAP. Keywords: LC3-associated phagocytosis; RAB5c; *Aspergillus fumigatus*.

2634 - Category: CELLULAR IMMUNOLOGY (CE)

Fast PBMC Isolation by Density Gradient: A Time-Saving and Reliable Alternative

DOS SANTOS, T.M.A.F.; CÂNDIDO, A.K.P.; MAZZINGHY, G.S.; SILVA, B.M.M.; VAZ, L.G.; SANTIAGO, H.D.C.; ALMEIDA, G.G.. CT- VACINAS/UFGM, BELO HORIZONTE - MG - BRASIL.

Peripheral blood mononuclear cell (PBMC) isolation via density gradient centrifugation is widely used in immunological assays. However, standard protocols are time-consuming, limiting sample throughput and increasing the risk of technical variability. **Objective:** To validate a rapid PBMC isolation protocol and compare its performance to the standard method regarding cell population frequencies and processing time. **Methods:** Blood samples from 15 healthy volunteers (n=5/day over 3 days) were processed in parallel using two protocols. The standard protocol consisted of centrifugation at $400 \times g$ for 40 minutes at room temperature with very slow deceleration. The rapid protocol used $1200 \times g$ for 10 minutes under the same conditions. After isolation, cells were cryopreserved in 10% DMSO, stored at -80°C for 24 hours, and transferred to liquid nitrogen. Upon thawing in culture medium with 1% Benzonase, samples were stained with antibodies anti CD3, CD4, CD8, CD14, CD16, CD19, CD45RO, CD56, CD66b, and CCR7, along with a viability stain. Flow cytometry analysis was performed using FlowJo, and statistical analysis in R. **Results:** No statistically significant differences were observed between protocols in total PBMC recovery or in the frequencies of T cell, B cell, NK cell, and monocyte populations. Functional T cell subsets (naïve, effector, central memory, effector memory), NK subsets (dim/bright), and monocyte subtypes were preserved across both methods. The rapid protocol reduced processing time by approximately 90 minutes without compromising cell viability or immunophenotypic profiles. **Conclusion:** The rapid PBMC isolation protocol is a viable alternative to the standard method, maintaining analytical reliability while significantly reducing processing time. Its implementation may enhance workflow efficiency and reduce variability in high-throughput immunological studies. Keywords: PBMC;Protocols;Flow cytometry.

2650 – Category: CELLULAR IMMUNOLOGY (CE)

Profile of Systemic Extracellular Vesicles in Symptomatic and Asymptomatic individuals infected by Plasmodium vivaxDE BROU, N.C.¹; SILVESTRINI, M.M.A.¹; COSTA, C.M.²; ALMEIDA, G.G.¹; PEREIRA, D.³; TADA, M.S.³; GAZZINELLI, R.T.¹; MARTINS-FILHO, O.A.¹; DE CARVALHO, A.T.¹; ANTONELLI, L.R.D.V.¹. 1. FIOCRUZ MINAS - INSTITUTO RENÉ RACHOU, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. CENTRO DE PESQUISA EM MEDICINA TROPICAL, PORTO VELHO - RO - BRASIL.

Malaria is an infectious disease with widespread distribution. In the Americas, *Plasmodium vivax* is the predominant species responsible for most cases. Infections can often be asymptomatic (ASY) and characterized by low parasitemia, but they are still able to transmit the parasite to vectors. Previous studies demonstrated that extracellular vesicles (EVs), particles released by all cell sources, are increased during symptomatic (SY) malaria. In this current study, we intend to better describe the EV profile during *Malaria vivax*. Our hypothesis is that SY, ASY and non-infected individuals (CTL) have distinct EV profiles, such as concentration, biogenesis, and cell sources. Peripheral blood samples were collected in tubes containing sodium citrate from individuals living in the municipalities of Candeias do Jamari and Porto Velho, Rondônia, Brazil. Six CTL and six SY were selected for the pilot study. The EVs were isolated using high-speed centrifugation. For immunophenotyping, the samples were incubated with monoclonal antibodies conjugated with fluorochromes to determine the cellular source of the EVs. Annexin V was used to identify total EVs. Samples were acquired on a CytoFlex S flow cytometer, and the data were analyzed using FlowJo and GraphPad Prism softwares. In addition, Nanoparticle Tracking Analysis (NTA) to estimate the concentration and average diameter of EVs in suspension. Preliminary data showed a higher percentage and mean absolute number of circulating EVs, with sizes ranging from 100 to 900 nm, in SY patients compared to CTL. The NTA results corroborate those obtained from immunophenotyping, showing a higher concentration of EVs in SY than in CTL. As perspectives, we will include ASY and additional samples from SY and CTL groups and assess molecules that indicate infection in cryptic sites such as liver, spleen, and bone marrow. The characterization of EVs may be a sensitive and non-invasive tool to support parasite detection in ASY patients. Keywords: Extracellular Vesicles;Plasmodium vivax;Asymptomatic.

2671 – Category: CELLULAR IMMUNOLOGY (CE)

Adoptive transfer of macrophages ameliorates chemotherapy-induced intestinal mucositis

MURTA, G.K.T.; DE SOUZA, I.A.; PAIXAO, P.H.D.M.; CALIARI, M.V.; GONÇALVES, R.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

5-Fluorouracil (5-FU) has been used as an effective chemotherapy agent for more than 40 years, but is considered a controversial drug because it causes serious side effects such as mucositis. Mucositis is an acute condition of the mucosa of the gastrointestinal tract, related to the damage of epithelial cells and inflammation resulting from the use of chemotherapy. It is associated with fatal complications that lead to a high impact on morbidity, mortality and the cost of cancer treatments. This reinforces that prevention of intestinal mucositis is important to improve the success of chemotherapy. However, currently available strategies focus on treating symptoms, and little have been explored in preventing the development of the disease. Macrophages are abundant cells in the intestine, with the ability to respond to the microenvironment and change their polarization to different profiles, in an attempt to promote intestinal homeostasis. As macrophages are cells involved in inflammatory response process and capable of producing molecules that act simultaneously on epithelial and other immune cells, we investigated whether intraperitoneal adoptive transfer of resting Bone Marrow-Derived Macrophages (BMDM) could affect the clinical and histomorphological characteristics of murine intestinal mucositis caused by 5-FU, focusing on a cell therapy strategy. Treatment with 5×10^6 macrophages promoted faster body weight recovery in mice, and showed that they were able to preserve the mucosal architecture of small intestines, not only when evaluated the measurement of villi and crypts heights, but also using histological parameters such as the degree of inflammation, vascular changes, mucosal atrophy and presence of inflammatory cells. Furthermore, macrophages contributed to the preservation of goblet cells and the protection of mucus layer by when evaluated by PAS+ morphometric assessment. Keywords: macrophages;intestinal mucositis;cell therapy.

2679 – Category: CELLULAR IMMUNOLOGY (CE)

TREATMENT WITH BACILLUS VELEZENSIS PRESERVED INTESTINAL BARRIER INTEGRITY IN MICE DURING DSS-INDUCED COLITIS

LIMA, A.C.; COELHO, M.M.; ALVES, J.L.; DUTRA, J.D.C.F.; AZEVEDO, V.; MAIOLI, T.U.; FARIA, A.M.C.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Introduction: Inflammatory bowel diseases (IBD) are gastrointestinal disorders marked by a disruption in intestinal homeostasis due to inflammatory immune responses to luminal antigens, such as those seen in colitis. Previous studies have demonstrated that probiotic can ameliorate disease parameters in DSS colitis models. Thus, the aim of the present study was to analyze the effects of the *Bacillus velezensis* 144 strain on DSS-induced ulcerative colitis in C57BL/6 mice.

Methods: Acute ulcerative colitis was induced in C57BL/6 mice by administering a 2% dextran sodium sulfate (DSS) solution for seven consecutive days. We analyzed the effects of the *B. velezensis* (1×10^9 CFU/mouse/day), which was administered by gavage for seven days concurrently with the DSS treatment. **Results:** Body weight loss was evident in DSS-treated mice, but treatment with *B. velezensis* 144 strain mitigated this effect. In addition, the probiotic treatment improved the overall clinical score of the disease, including factors such as diarrhea, weight loss, and blood in the feces. Notably, it prevented the reduction in colon length associated with DSS-induced colitis. Supporting these data, we observed a reduction in the histologic score, which includes parameters such as destruction of normal mucosal architecture, degree of cellular infiltration, extent of muscle thickening, presence of crypt abscesses, and goblet cell depletion. Additionally, there was an increase in mucus production and increased number of regulatory T cells in the *B. velezensis*-treated groups, indicating a modulation of the immune response. **Conclusion:** We conclude that *B. velezensis* ameliorated the clinical outcomes of DSS-induced colitis and preserved the integrity of the intestinal barrier in this disease model. Keywords: intestinal mucosa;IBD;probiotics.

2695 – Category: CELLULAR IMMUNOLOGY (CE)

The influence of calcineurin in modulating *Trypanosoma cruzi* infection in RAW 264.7 macrophages

PEREIRA, C.L.¹; DA SILVA, L.E.M.²; DA SILVA, C.V.¹; DE CARVALHO, G.M.S.¹; SILVEIRA, A.C.A.²; DE OLIVEIRA, E.C.M.¹; VELIKKAKAM, T.¹; FORTUNATO, C.E.S.¹. 1. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, UBERLÂNDIA - MG - BRASIL.

Calcineurin is a phosphatase involved in the activation of immune signaling pathways. This study aimed to investigate the role of calcineurin in *Trypanosoma cruzi* infection using RAW 264.7 macrophages treated with cyclosporine A, a pharmacological inhibitor of calcineurin. *In vitro* assays were conducted to assess parasite invasion and intracellular multiplication, as well as nitric oxide production and cytokine levels (IL-1 β , IL-10, IL-12, and IL-6) by ELISA. Experimental groups included: untreated control, pre-treatment (1 hour before infection), post-treatment (drug administered after infection), and combined treatment (pre-treatment followed by continuous exposure). Calcineurin inhibition with cyclosporine A reduced the cell invasion rate and intracellular multiplication of the parasite, particularly in the pre-treatment and post-treatment groups. Immune modulation was evidenced by increased levels of IL-1 β and IL-10, and decreased levels of IL-12. Nitric oxide production was also altered, with reduced release observed in the treated groups, suggesting a shift in the functional profile of macrophages. These findings highlight the role of calcineurin in regulating the innate immune response and suggest that its inhibition may help control *T. cruzi* infection by modulating the cellular microenvironment and cytokine production. Keywords: Chagas disease; Calcineurin; immunomodulation.

2696 – Category: CELLULAR IMMUNOLOGY (CE)

Interplay Between Cell Death Pathways and Inflammatory Response in *Staphylococcus aureus*-Induced Pneumonia in Balb/c Mice

FORTUNATO, C.E.S.¹; PEREIRA, C.L.¹; SILVEIRA, A.C.A.¹; DE OLIVEIRA, E.C.M.¹; VELIKKAKAM, T.¹; CHIVALE, N.L.¹; DE CARVALHO, G.M.S.¹; DA SILVA, C.V.¹; CASSEL, M.². 1. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO, ITURAMA - MG - BRASIL.

Bacterial pneumonia is often caused by *Staphylococcus aureus*, a commensal organism capable of proliferating in the respiratory tract and releasing toxins, thereby triggering inflammation and cell death. This study evaluated histopathological changes and disease progression in BALB/c mice exposed to increasing concentrations of *S. aureus* (10^7 to 10^9 CFU). Histological (H&E) and immunohistochemical (peroxidase) analyses were performed to assess inflammatory markers (TNF- α , NF- κ B, IL-6) and cell death markers (Bcl-2, Bax, Caspase-3, Cathepsin-D). Observed alterations included edema, bronchiolar and vascular disruption, alveolar collapse, and bronchiolar hyperplasia, with the latter showing a significant difference between the 10^7 and 10^8 CFU groups. Apoptosis was predominant at lower bacterial concentrations ($\leq 10^8$ CFU), whereas autophagy and reduced inflammatory markers were more prominent at the highest dose (10^9 CFU). This suggests a shift toward cellular preservation during severe infection, as supported by histological analysis. These findings highlight the dynamic balance between inflammation and cell death in *S. aureus*-induced pneumonia. Keywords: Immunohistochemistry; Inflammation; Cell death.

2697 – Category: CELLULAR IMMUNOLOGY (CE)

Immunoregulation mediated by the P21 protein in intracellular amastigotes of *Trypanosoma cruzi*

SILVEIRA, A.C.A.¹; TEIXEIRA, T.L.²; PEREIRA, C.L.¹; DE OLIVEIRA, E.C.M.¹; CHIVALE, N.L.¹; FORTUNATO, C.E.S.³; VELIKKAKAM, T.¹; DA SILVA, C.V.¹. 1. UFU, UBERLÂNDIA - MG - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL; 3. UFU, UBERLÂNDIA - MG - BRASIL.

The P21 protein of *Trypanosoma cruzi* is known for modulating the host immune response. In this study, we analyzed by RNA sequencing (RNAseq) the gene expression profile in intracellular amastigotes from Y and G strains with a P21 gene deletion (TcP21^{-/-}), to investigate the impact of this protein's absence and its potential immunoregulatory consequences. We observed differential modulation of genes related to processes such as cell adhesion, protein phosphorylation, translation, and oxidative stress regulation. Notably, a significant modulation in the expression of genes encoding tryparedoxin peroxidase was detected in both strains. This enzyme is crucial for neutralizing reactive oxygen species, essential for parasite survival within the intracellular environment and for immune evasion. The upregulation of tryparedoxin peroxidase in TcP21^{-/-} parasites suggests a compensatory mechanism in response to the loss of a protein with known immunomodulatory functions. Additionally, the downregulation of genes involved in cell adhesion and glycosylation in the Y strain suggests impaired host-parasite interaction, which may affect the resulting immune response. These findings indicate that the P21 protein may influence immunoregulation during infection by modulating the expression of genes involved in both parasite survival and the establishment of chronic infection. Keywords: *Trypanosoma cruzi*; tryparedoxin peroxidase; immunoregulation.

2700 – Category: CELLULAR IMMUNOLOGY (CE)

Modulation of cytokine secretion by macrophages infected by different phylogenetic lineages of *Trypanosoma cruzi*

DE CARVALHO, G.M.S.; PEREIRA, C.L.; DA SILVA, L.E.M.; DA SILVA, C.V.; SILVEIRA, A.C.A.; DE OLIVEIRA, E.C.M.; VELIKKAKAM, T.; FORTUNATO, C.E.S.; CHIVALE, N.L.. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL.

The genetic diversity of *Trypanosoma cruzi* can directly influence the host immune response. In this study, we evaluated how strains belonging to different Discrete Typing Units (DTUs) modulate macrophage activation. Trypomastigote forms of strain G (DTU I) and strain Y (DTU II) were used in single and mixed infections of RAW 264.7 macrophages. Parasite invasion and intracellular multiplication assays were conducted to compare the behavior of the two strains. Culture supernatants were collected at two time points—24 and 96 hours post-infection—and analyzed by ELISA to quantify the cytokines IL-6, IL-10, IL-12, IL-18, and IL-1 β . Strains G and Y of *T. cruzi* induced distinct cytokine secretion profiles, consistent with their genetic differences and infectivity. The observed variability in cytokine responses suggests that *T. cruzi* genetic diversity influences macrophage activation during both single and co-infections. The differentiation of cytokine profiles in co-infections indicates the potential for unique immune modulation patterns, which may have implications for the pathogenesis and clinical progression of Chagas disease. Keywords: Cytokines; *Trypanosoma cruzi*; ELISA.

2703 – Category: CELLULAR IMMUNOLOGY (CE)

Immune Response Modulation in Co-infection with *Trypanosoma cruzi* and *Leishmania (L.) amazonensis* In Vitro and In Vivo

CHIVALE, N.L.; DE OLIVEIRA, E.C.M.; SILVEIRA, A.C.A.; PEREIRA, C.L.; VELIKKAKAM, T.; FORTUNATO, C.E.S.; BANZE, L.R.; DA SILVA, C.V.. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL.

American trypanosomiasis and leishmaniasis are parasitic diseases that frequently coexist in Latin America, leading to co-infections with poorly understood impacts on immune responses. This study aimed to investigate immune modulation during coinfection with *Trypanosoma cruzi* and *Leishmania (L.) amazonensis* using in vitro and in vivo models.

In vitro, bone marrow-derived macrophages (BMDMs) were used to assess parasite replication, and RAW 264.7 cells were used to quantify parasite load (qPCR) and cytokine profiles (ELISA). In vivo, BALB/c mice were infected with *T. cruzi*, *L. amazonensis*, or both. Heart, paw, blood, spleen, and liver samples were collected at days 7, 15, and 30 post-infection. Cardiac histopathology, parasite burden, and serum cytokines were evaluated.

T. cruzi showed higher invasion and replication in BMDMs than *L. amazonensis*. *T. cruzi* load was consistently greater in all conditions. In contrast, *L. amazonensis* load remained stable and low across groups. At 72h and 96h post-infection, coinfecting cultures had lower *T. cruzi* load than single infections, suggesting interference by *L. amazonensis*.

In vivo, coinfection worsened *T. cruzi*-induced cardiac inflammation and increased its burden in the heart and spleen. While *L. amazonensis* was undetectable in visceral organs, it showed increased presence in the paw of coinfecting animals, suggesting localized inter-parasite modulation.

These findings indicate that *T. cruzi* dominates infection but that *L. amazonensis*, despite its low burden, can affect *T. cruzi* replication and inflammatory responses in a tissue-specific and complex manner. Further analyses of inflammasome pathways and cytokines are ongoing. Keywords: *Trypanosoma cruzi*; Immune Response Modulation; *Leishmania amazonensis*.

2704 – Category: CELLULAR IMMUNOLOGY (CE)

Immunological Modulation and Differential Vesicular Trafficking in Experimental *Trypanosoma cruzi* Infection via Oral and Subcutaneous Routes

DE OLIVEIRA, E.C.M.; PEREIRA, C.L.; SILVEIRA, A.C.A.; BARBOSA, E.R.M.D.C.; FORTUNATO, C.E.S.; CHIVALE, N.L.; BANZE, L.R.; TORRES, J.D.A.; MOTA, C.M.; MINEO, T.W.P.; DA SILVA, C.V.. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL.

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, can be transmitted through multiple routes, each capable of distinctly modulating the host immune response. This study compared the immunomodulatory effects of subcutaneous (SC) and oral (OR) infection routes in BALB/c mice experimentally infected with the Y strain of *T. cruzi*. At 30 days post-infection (dpi), gene expression analysis, cytokine profiling, and histopathological evaluation were performed. OR infection induced the expression of Arginase, LAMP-1, and YM1 — genes associated with M2 macrophage polarization and anti-inflammatory responses — alongside a significant increase in serum IL-18 levels, indicating an immune profile influenced by mucosal immunity. In contrast, SC infection led to elevated levels of IL-6, MCP-1, and TNF- α , pro-inflammatory cytokines linked to cardiac inflammatory infiltrates and potential chronic tissue damage. Both infection routes modulated genes involved in vesicular trafficking: upregulation of RAB5 and RAB7, and downregulation of Syntaxin 18 (SYT18), suggesting enhanced phagolysosomal and endosomal activity, potentially affecting parasite internalization and antigen presentation. Additionally, Cathepsin D was downregulated in both groups, with more pronounced suppression in the SC group, which may impair lysosomal degradation and antigen processing. The reduction of SYT18 may also impact ER-Golgi trafficking and cytokine secretion. Overall, the infection route distinctly modulates immune pathways, with important implications for the pathogenesis and outcome of *T. cruzi* infection. Keywords: *Trypanosoma cruzi*; Chagas disease; Immunomodulation.

2718 – Category: CELLULAR IMMUNOLOGY (CE)

Frequency of CTLA-4+ Lymphocytes Incubated in the Presence and Absence of Tumor Lysate from MDA-MB-231 Cells

OLIVEIRA, A.V.C.; FREITAS, B.A.D.A.; COSTA, M.A.; SOUZA, I.D.F.; TORRES, L.A.G.. FEDERAL UNIVERSITY OF THE JEQUITINHONHA AND MUCURI VALLEYS (UFVJM), DIAMANTINA - MG - BRASIL.

Triple-negative breast cancer (TNBC) is an aggressive form of the disease, characterized by the absence of estrogen, progesterone, and HER2 hormone receptors, which limits therapeutic options and makes chemotherapy the main treatment approach. In the context of tumor pathophysiology, an immunosuppressive immune microenvironment is observed, with a predominance of exhausted phenotype T lymphocytes, immunosuppressive cytokines, and M2 subtype macrophages, all of which contribute to the tumor's immune evasion. This study aimed to evaluate the frequency of CTLA-4+ lymphocytes incubated in the presence and absence of tumor lysate from MDA-MB-231 cells. This project was approved by the Ethics and Research Committee of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM), approval number 7.548.477. For this purpose, PBMCs were incubated for five days with lysate from MDA-MB-231 cells, a cell line representative of TNBC, in 24-well plates containing RPMI medium supplemented with L-glutamine, fetal bovine serum, antibiotic, and antifungal. After the incubation period, the cells were stained with an anti-CTLA-4 antibody conjugated with PE and analyzed by flow cytometry using a FACSCan device, acquiring 10,000 events within the lymphocyte gate. The results showed an approximately sixfold increase in the frequency of CD28+ cells in the samples stimulated with tumor lysate compared to the unstimulated samples. These findings suggest that tumor lysate may interfere with lymphocyte activation, highlighting the challenge of strategies aimed at reversing the cellular exhaustion state observed in the tumor microenvironment. Further studies are needed to assess whether prior activation with tumor lysate contributes to a more effective antitumor response. Keywords: Triple Negative Breast Neoplasms; T-Lymphocytes; tumor lysate.

2733 – Category: CELLULAR IMMUNOLOGY (CE)

Ethanol-treated human fibroblasts show alterations in mitochondrial metabolism and modulate CD4+ T lymphocyte activation

DE FIGUEIREDO, D.; BUSO-LOPES, A.F.; DE ROSSI, T.; CRUZ, R.G.B.; CARDOSO, E.C.; TAMARINDO, G.H.; FARIA, J.M.D.L.; SÁ, J.D.O.; LEME, A.F.P.. CNPEM, CAMPINAS - SP - BRASIL.

Purpose: Oral squamous cell carcinoma (OSCC) may arise due to various risk factors, including tobacco and alcohol consumption. However, the mechanism by which alcohol contributes to OSCC initiation remains to be elucidated. **Methods:** Fibroblasts from normal tissue were treated with ethanol (EtOH) (n=3) to assess oxygen consumption rate, mitochondrial superoxide production, and mitochondrial morphology by transmission electron microscopy. Peripheral blood mononuclear cells (PBMCs) from healthy donor buffy coats were co-cultured with both treated and untreated fibroblasts and their extracellular vesicles (EVs). T cell activation was analyzed by measuring CD25 and CD69 expression at both mRNA and protein levels using RT-qPCR (n=5) and flow cytometry (CD25, n=8; CD69, n=5), respectively. Cytometric bead arrays were used to evaluate cytokines released into the culture supernatants (n=8). **Results:** EtOH-treated fibroblasts showed an increase in proton leak (p=0.0290, paired Student's t-test), mitochondrial superoxide production (p=0.0386, paired Student's t-test), and mitochondrial alterations such as a diffuse matrix, disorganized cristae, and regions of membrane discontinuity, indicating mitochondrial dysfunction. PBMCs co-cultured with EtOH-treated fibroblasts exhibited lower CD69 mRNA expression (p=0.0245; paired Student's t-test) and a decrease in the percentage of CD4+ T cells expressing CD25 (p=0.0156; Wilcoxon test) and CD69 (p=0.0417; paired Student's t-test). PBMCs co-cultured with EVs from EtOH-treated fibroblasts showed increased expression of CD25 (p=0.0327, paired Student's t-test), and in the supernatants from this culture, increased levels (Wilcoxon test) of IL-6 (p=0.0313), TNF- α (p=0.0391), and IL-10 (p=0.0469). **Conclusion:** EtOH-treated fibroblasts exhibit altered mitochondrial metabolism and modulate T cell activation and the cytokine release profile in culture supernatants. FAPESP supported this work under grant numbers 2018/18496-6 and 2022/12815-8. Keywords: Fibroblasts; T cell activation; Mitochondrial dysfunction.

2735 – Category: CELLULAR IMMUNOLOGY (CE)

Impact and Safety of Probiotic Supplementation in Intestinal Inflammatory Conditions

RODRIGUES, G.M.B.¹; GONÇALVES, L.M.¹; DA SILVA, G.W.¹; DE ARAUJO, M.V.¹; OLIVEIRA, B.D.C.¹; ALVES, C.B.¹; DE ABREU, A.M.¹; DA CONCEIÇÃO, L.B.¹; CARDOSO, A.¹; MARTINS, F.D.S.²; DA FONSECA, D.M.¹. 1. LABORATORY OF MUCOSAL IMMUNOLOGY, ICB IV - USP/NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY FOR RESEARCH IN INFECTIOUS AND CHRONIC DISEASES OF THE MUCOSA AND SKIN - INCT MUCOSA AND SKIN, SÃO PAULO - SP - BRASIL; 2. LABORATORY OF BIOTHERAPEUTIC AGENTS, ICB - UFMG, SÃO PAULO - SP - BRASIL.

The intestinal mucosa is constantly exposed to antigens, such as pathogens, innocuous dietary, and commensal microbiota-derived antigens, which influence homeostasis. In the Laboratory of Mucosal Immunology, we study how the exposure of gut mucosa to microorganisms, such as probiotics or pathogens, can shape the gut-associated mucosal immune system. We observed that after the resolution of *Yersinia pseudotuberculosis* (YP) infection, there is a remodeling of the immune and lymphatic systems of the gastrointestinal tract, which leads to microbiota translocation to the mesentery, resulting in chronic local inflammation. This process, which we have named “immunological scarring,” predisposes to the development of severe forms of colitis. Therefore, preventing the immunological scar could hinder this process. Literature indicates that in a model of mucositis, *Saccharomyces cerevisiae* (SC) UFMG A-905 treatment reduces intestinal permeability and preserves tissue architecture. We hypothesized that the previous treatment with this probiotic could recover the integrity of the mesenteric lymphatic system, and thus, the migratory capacity of the CD103+ dendritic cells (DCs). However, SC UFMG A-905 treatment, whether previous to or post-YP infection, showed no promising results. On the contrary, it worsened the chronic inflammation caused by the infection. Increasing the frequency of Th1 lymphocytes and Innate Lymphoid Cells 1 (ILC1) in the mesentery, the mesenteric lymphadenopathy was also increased in comparison to infected and non-treated animals. Additionally, the treatment was unable to restore the regulatory response in the mesentery, as the T regulatory cells (Tregs), particularly the Treg RORγt+ population, showed no increase after treatment in infected animals. Until now, we concluded that the beneficial effect of probiotics treatment may be contextual, and that its consumption during intestinal inflammatory processes might be dangerous. Keywords: Immunological scar; Probiotics; Infection.

2741 – Category: CELLULAR IMMUNOLOGY (CE)

Effect of ketogenic diet on the development of experimental asthma

ALVES, C.B.; DE ABREU, A.M.; DA CONCEIÇÃO, L.B.; DE ASSIS, J.B.; DA SILVA, G.W.; CARDOSO, A.; DE ARAUJO, M.V.; RODRIGUES, G.M.B.; GONÇALVES, L.M.; OLIVEIRA, B.D.C.; COELHO, M.P.S.; CAMARGO, L.; AYUPE, M.C.; DA FONSECA, D.M.. LABORATORY OF MUCOSAL IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES - USP / NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY FOR RESEARCH IN INFECTIOUS AND CHRONIC DISEASES OF THE MUCOSA AND SKIN, SÃO PAULO - SP - BRASIL.

The mucosa-associated immune system is constantly challenged by external factors such as infections, allergen exposure, microbiota colonization and dietary changes, which can disrupt its homeostasis. Recently, there has been a rise in the consumption of unbalanced diets such as the ketogenic diet (KTD), commonly known as “low-carb diet”, by healthy individuals, which remains poorly explored. Known immunological interactions between mucosal tissues, such as the gut-lung axis, suggest that the effects of these disturbances, including the ketogenic diet itself, extend beyond local intestinal effects and reach the lung tissue. This can be mediated by dietary components, changes in the microbiota, or immune system cells and molecules. Our group has previously demonstrated that the consumption of the ketogenic diet led to pulmonary neutrophilia, which exacerbated inflammatory lung diseases, like COVID-19. Given this, we hypothesized that allergic asthma, as an inflammatory disease, could also have its progression altered by KTD consumption, potentially worsening the allergic response or shifting into a neutrophilic asthma phenotype. However, the results so far reveal that KTD consumption actually reduces the development of asthma, as evidenced by a decrease in eosinophilic inflammatory infiltrates in the lungs (analyzed by bronchoalveolar lavage count and flow cytometry of lung tissue) without promoting a neutrophilic asthma profile. We subsequently found that this protective phenotype persists even when the diet is discontinued before asthma induction, suggesting that the effect may be mediated by long-lasting modifications in the gut-lung axis, such as alterations in both gut and lung microbiota. Therefore, this demonstrates that, although unbalanced diets may show certain beneficial effects in healthy individuals, these effects are highly context-dependent and could potentially lead to adverse outcomes. Keywords: ketogenic diet; allergic asthma; gut-lung axis.

2750 - Category: CELLULAR IMMUNOLOGY (CE)

PGC1 α -mediated mitochondrial fitness promotes Treg cell differentiation

DAMASCENO, L.E.A.¹; BARBOSA, G.A.D.C.¹; SPARWASSER, T.D.²; CUNHA, T.M.¹; CUNHA, F.Q.¹; ALVES-FILHO, J.C.¹. 1. UNIVERSITY OF SAO PAULO (FMRP-USP), RIBEIRÃO PRETO - SP - BRASIL; 2. INSTITUTE OF MEDICAL MICROBIOLOGY AND HYGIENE, UNIVERSITY MEDICAL CENTER OF THE JOHANNES GUTENBERG-UNIVERSITY, MAINZ - ALEMANHA.

T_{reg} cells play an important role in the maintenance of immunologic tolerance and the control of immune responses. Different metabolic pathways drive the phenotype of T_{reg} cells, which require mitochondrial integrity and metabolism to exert their functions. PGC1 α is a master transcriptional coactivator regulating mitochondrial biogenesis, dynamics, and oxidative metabolism in high-energy-demand tissues. Although low expression of PGC1 α has been linked to enhanced inflammation, little is known about its intrinsic role in immune cells. This work explores how PGC1 α connects the regulation of cellular metabolism to T_{reg} cell biology. For this, murine and human T_{regs} were generated (TGF β ; IL-2) from magnetically purified naïve CD4 T cells in the presence or absence of a PGC1 α activator(ZLN005) or inhibitor(SR18292), and subsequently analyzed for mitochondrial fitness, iT_{reg} differentiation levels, and suppressive phenotype. Statistical significance (p<0.05) was assessed by one- or two-way ANOVA with Tukey's post-test, or unpaired t-test, as appropriate; data shown as mean $\pm$ SEM. Our results revealed that high mitochondrial content is associated with enhanced iT_{reg} cell generation, and these cells exhibit a marked increase in PGC1 α gene and protein expression compared with naïve or TCR-only activated cells. Remarkably, pharmacological activation of PGC1 α was accompanied by increased mitochondrial content and mtDNA levels, as well as enhanced oxidative mitochondrial metabolism in iT_{reg} cells. In parallel, PGC1 α activation enhanced iT_{reg} differentiation, as evidenced by increased Foxp3⁺CD25⁺ population, and upregulated the expression of immunosuppressive molecules (PDL1, GARP), whereas PGC1 α inhibition reduced iT_{reg} generation. In a translational approach, PGC1 α activation enhanced both mitochondrial fitness and differentiation of human iT_{regs}. These findings shed light on the PGC1 α potential as a pharmacological target when manipulating T_{reg} cells as a therapeutic strategy. Keywords: PGC1 α ;Mitochondria;Treg cells.

2756 - Category: CELLULAR IMMUNOLOGY (CE)

VITA Monitoring: Characterization of markers in patients with post-covid conditions and their correlation with severity, persistence, and therapeutic evolution

LIMA, A.E.D.S.¹; LOURENÇO, A.J.¹; FILGUEIRAS, P.S.¹; OLIVEIRA, L.A.R.¹; CONTIN, S.V.M.¹; CAMARGOS, I.R.¹; MAGALHÃES, V.R.M.¹; DA SILVA, L.G.¹; CORRÊA, P.R.L.²; GOMES, M.F.D.C.³; QUEIROZ, R.F.G.E.¹. 1. OSWALDO CRUZ FOUNDATION - FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. BELO HORIZONTE CITY HALL, BELO HORIZONTE - MG - BRASIL; 3. MINISTRY OF HEALTH, BRASÍLIA - DF - BRASIL.

Some patients who have recovered from covid-19 have reported persistent symptoms for at least four weeks after infection, which have become known as post-covid conditions. Immune dysregulation is known to play a role in these conditions, although its exact mechanisms require further investigation. The study aimed to characterize immunological, hematological, and tissue damage markers after covid-19 and correlate with severity and symptom persistence. Participants included adults and children (>5 years old) from Belo Horizonte, MG, who reported ongoing post-covid conditions. Data were collected through an online form, after which selected individuals attended two clinical visits, spaced one month apart, for clinical evaluation and collection of biological samples. Participants connected to treatment services through the BH Municipal Health Department. The analysis included complete blood counts, a 27-marker multiplex assay via flow cytometry, and the assessment of general and cardiac tissue damage markers, coagulation, inflammation, oxidative stress, ferritin levels, and viral protein levels using ELISA. A total of 212 volunteers were enrolled, predominantly female and over 18 years old. Most had mild cases (89.2%) and up to five infections. The most frequent symptoms were memory impairment (69.3%) and fatigue (34.9%), with 44.8% reporting three to four symptoms. Common post-infection comorbidities included hypertension and anxiety. During follow-up, some chemokines and inflammatory cytokines showed an increase, which was concomitant with regulatory cytokines and growth factors. In contrast, D-dimer, red blood cells, hematocrit, and lymphocytes showed a decrease. Post-covid conditions are not limited to the respiratory system, underscoring the impact of the pandemic. Even with the increase in inflammation, the regulatory response also increased, showing a balance. These findings highlight the importance of continuous monitoring of these conditions. Keywords: post-covid conditions;biomarkers;immune response.

2757 - Category: CELLULAR IMMUNOLOGY (CE)

In vitro validation of potential therapeutic targets for T-cell acute lymphoblastic leukemia.

DE OLIVEIRA, R.S.¹; DA SILVA, L.L.F.¹; CARELS, N.²; CARNEIRO, F.R.G.². 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

T-cell acute lymphoblastic leukemia (T-ALL) accounts for 12–15% of childhood ALL and 25% of adult cases, representing an aggressive and heterogeneous disease. Identifying novel therapeutic targets and drugs to reduce treatment-related side effects is crucial. This study aims to uncover such targets by identifying proteins both overexpressed and highly connected in cellular networks. We analyzed RNA-seq data from peripheral T lymphocytes and healthy thymocytes as controls, along with T-ALL cell lines Jurkat and Molt-4. Normalized expression data from each control group were independently compared with those from the T-ALL cell lines to identify overexpressed genes in malignant cells. The interactome from the European Bioinformatics Institute was used to calculate the number of interactions per upregulated gene and to prioritize those with the highest connectivity. Most identified genes were consistent across both cell lines, regardless of the type of control used for comparison. Candidate targets included oncogenes, chaperones, regulatory proteins, and proteins involved in metabolism, autophagy, and cell division. Notably, NPM1 and CDC5L were highlighted. NPM1 encodes nucleophosmin, a protein involved in centrosome duplication, histone assembly, ribosome biogenesis, and tumor suppressors regulation. CDC5L positively regulates cell cycle progression and participates in pre-mRNA splicing. Overexpression of both genes was confirmed in Jurkat and Molt-4 cells via RT-qPCR and western blot. To validate their roles in vitro, knockdown experiments using shRNA lentiviral particles were performed, followed by phenotypic analysis. Cell proliferation and viability were assessed using trypan blue exclusion and MTT assays over a 96-hour time course, showing reduced proliferation and viability. These findings suggest that NPM1 and CDC5L are crucial for cell division and survival in T-ALL, supporting our bioinformatics-driven approach for identifying viable therapeutic targets. Keywords: Leukemia;T-ALL;Bioinformatics.

2758 - Category: CELLULAR IMMUNOLOGY (CE)

Effects of Digoxin on lymphocyte populations and in the murine melanoma model (B16F10)

CAMPOS, M.L.A.¹; SILVA, M.C.S.¹; SAMPAIO, M.S.D.A.¹; TEIXEIRA, M.P.²; DA COSTA, E.V.¹; MASCARENHAS, G.D.³; DIAZ, B.L.³; DE ARAUJO, E.G.¹; CABRAL, V.R.¹; DE PAIVA, L.S.¹. 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL; 2. UNIVERSIDAD NACIONAL DE CÓRDOBA, CÓRDOBA - ARGENTINA; 3. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Digoxin is a cardiotonic glycoside that inhibits the Na⁺/K⁺ ATPase pump. While its cardiovascular actions are well characterized, its immunomodulatory properties, particularly on B lymphocytes, remain insufficiently explored. Previous studies have reported its anti-inflammatory and antitumor effects. Melanoma is the most aggressive type of skin cancer, with limited treatment success. Thus, repurposing approved drugs like Digoxin may offer promising therapeutic opportunities. In this study, we evaluated the immunomodulatory effects of Digoxin in two contexts: immune homeostasis and in the syngeneic melanoma model (B16F10). In the homeostatic model, Balb/c mice received i.p injections of Digoxin (0,3125mg/Kg/day) or vehicle for three consecutive days. In the melanoma model, C57BL/6 mice were pretreated intraperitoneally with Digoxin or vehicle prior to s.c. inoculation with B16F10 melanoma cells. Euthanasia was performed twenty-four hours after the last injection in both models. Digoxin significantly increased the absolute number of B cells in the spleen and mesenteric lymph nodes, but this effect was not dependent on increased proliferation, viability or activation status. An increase in serum of total IgM and IgG were also observed. Despite the increase in the number of B lymphocytes in the spleen, there was a reduction in the expression of ERK1/2 in this organ, suggesting that this effect is independent of this proliferation pathway. In vitro, Digoxin decreased secretion of cytokines IL-17, IL-6, and IL-2, indicating an anti-inflammatory profile. In the tumor model, mice receiving Digoxin exhibited increased percentages of CD4⁺ and CD8⁺ T cells in the spleen and in the number B lymphocyte in the tumor-draining lymph node, compared to untreated tumor-bearing controls. These data suggest that Digoxin modulates immune responses differently depending on the physiological or tumoral context and highlight its potential for therapeutic repurposing in melanoma. Keywords: Digoxin;B lymphocytes;immunomodulation.

2769 - Category: CELLULAR IMMUNOLOGY (CE)

Effect of Different Freezing Media on the Viability of Dendritic Cells for Use in Immunotherapy

VALE, L.B.¹; ORTEGA, M.M.²; MOTA, S.D.J.²; SUMIDA, T.M.O.². 1. UNIVERSIDADE NOVE DE JULHO, SÃO PAULO - SP - BRASIL; 2. FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Introduction: Dendritic cell (DC) based immunotherapy aims to enhance the immune response of affected individuals by promoting antigen presentation and clonal expansion of antigen-specific lymphocytes. Maintaining cell viability, from precursor cells to the final product, is essential for the success of immunotherapy, which requires efficient cryopreservation protocols under good manufacturing practices (GMP) conditions. **Objectives:** Evaluate the effect of fetal bovine serum (FBS) free freezing solutions on the preservation of peripheral blood mononuclear cells (PBMCs) and assess the final product in terms of viability, cell yield, and phenotypic profile of DCs differentiated from cryopreserved PBMCs. **Methods:** PBMCs were frozen using three different media: (1) a standard solution composed of FBS and dimethyl sulfoxide (DMSO); (2) a solution of DEXTRAN 40 and DMSO; and (3) the commercial solution CryoStor® CS10. After thawing, PBMCs were assessed for viability and were cultured under a dendritic cell differentiation protocol. The resulting cells were phenotypically analyzed concerning CD11c, CD14, HLA-DR, CD86 and CCR7 molecules expressed by flow cytometry. **Results:** Regarding the viability of precursor cells (PBMCs), a significant reduction was observed in samples preserved with DEXTRAN compared to those in the standard freezing medium (with FBS). Notably, all three media showed viability rates above 80%. For differentiated α -DC1 cells, no significant difference in viability was found, however, the yield of immature α -DC1 was significantly lower in samples preserved with DEXTRAN than in those with FBS. No significant differences were observed in the phenotypic profiles of differentiated α -DC1 cells. **Conclusion:** The viability of PBMCs and the yield of α -DC1 were lower in samples preserved with the DEXTRAN based medium compared to the standard freezing medium. Samples preserved with CryoStor® CS10 showed similar results to the standard medium. Keywords: immunotherapy;dendritic cells; cryopreservation.

2772 - Category: CELLULAR IMMUNOLOGY (CE)

HIF-1A IS REGULATED BY PARKIN AND INFLUENCES MITOPHAGY IN INFLAMMATORY MACROPHAGES

DOS SANTOS, T.P.¹; MARTINS, A.J.E.¹; DA SILVA, G.R.¹; ANGELIM, M.K.S.C.²; CASTELUCCI, B.G.¹; BARBOSA, M.¹; SANTOS, W.G.S.¹; DAVANZO, G.G.³; CUNHA, L.D.⁴; VIEIRA, P.M.M.D.M.¹. 1. UNIVERSIDADE ESTADUAL DE CAMPINAS (UNICAMP), CAMPINAS - SP - BRASIL; 2. SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE, ST. LOUIS - ESTADOS UNIDOS DA AMERICA; 3. WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, ST. LOUIS - ESTADOS UNIDOS DA AMERICA; 4. UNIVERSIDADE DE SÃO PAULO (USP), RIBEIRÃO PRETO - SP - BRASIL.

Macrophages undergo metabolic changes to respond to different stimuli. *In vitro*, macrophages activated with LPS and interferon- γ (LPS/ γ) show increased glycolysis, while mitochondrial respiration is impaired, with elevated membrane potential and increased mitochondrial ROS (mtROS) production. In various cell types, mtROS acts as a signal to induce the removal of dysfunctional mitochondria via the mitophagy pathway. In LPS/ γ macrophages, mtROS is also involved in the stabilization of hypoxia-inducible factor 1 α (HIF-1 α). HIF-1 α induces the expression of BNIP3, one of the key factors in the non-canonical mitophagy pathway. However, the role of HIF-1 α in modulating mitophagy in LPS/ γ macrophages remains unclear. Thus, we propose to elucidate the role of HIF-1 α in the regulation of mitophagy in inflammatory macrophages. Our data indicate that HIF-1 α -/- macrophages display higher numbers of autophagosomes and lysosomes, along with increased mitochondrial mass after stimulation with LPS/ γ . Additionally, the expression of lysosomal cathepsins is reduced in HIF-1 α -deficient macrophages, suggesting that an alteration in the lysosomal compartment may be affecting mitochondrial degradation. Next, we evaluated the macrophage profile in a context of reduced mitophagy. Parkin deletion in LPS/ γ macrophages led to decreased levels of HIF-1 α and BNIP3, indicating that HIF-1 α is regulated by Parkin and that PARKIN deletion inhibits both canonical and non-canonical mitophagy pathways. IL-10, TNF- α , and IL-6 levels were also reduced in Parkin-deficient LPS/ γ macrophages. These macrophages exhibited increased bactericidal capacity along with enhanced production of reactive oxygen species (ROS). Taken together, these data indicate that HIF-1 α regulates and is regulated by mitophagy in inflammatory macrophages, which directly impact their functionality. Keywords: Macrophage;Mitochondria;Mitophagy.

2796 - Category: CELLULAR IMMUNOLOGY (CE)

MACROPHAGE INTERACTION WITH HTLV-1-INFECTED CELLS INDUCED ALARMIN PRODUCTION

PIMENTA, J.V.; CAMPOS, M.C.S.; DUTRA, F.F.; LIMA, J.E.N.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

The human T-cell lymphotropic virus type-1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis is a neurodegenerative disorder with progressive loss of motor function, which can lead to paraplegia. Elevated levels of CXCL10, IFN γ , and TNF α characterize the inflammatory microenvironment and are associated with glial cell stress, demyelination, and axonal damage. HTLV-1 primarily infects CD4⁺ T cells, but it can also infect CD8⁺ T cells, dendritic cells, monocytes, and macrophages. During infection, certain endogenous molecules known as alarmins, or damage-associated molecular patterns, can be released by the host. The alarmin tryptophanyl-tRNA synthetase (WARS) is a protein that plays a non-canonical role in the activation of inflammatory processes elicited by viruses. We aimed to investigate the effects of macrophage (M ϕ) interaction with HTLV-1-infected cells, focusing on the expression of WARS. For this, THP1 monocytes were differentiated into M ϕ s using PMA (100 ng/mL) for 72h. Then, M ϕ (2x10⁶ cells/well) were co-cultured with a cell line infected by HTLV-1 (MT2: 2.5 or 5x10⁵ cells/well). After 18h, the supernatant was removed and replaced with fresh culture medium for an additional 6h. The results obtained by Western Blot indicated a significant increase in WARS levels in both the co-culture supernatant (~3X) and cell extracts (~2X), suggesting enhanced production and secretion. Preliminary results obtained by ELISA indicate that CXCL10 levels in the co-culture supernatants are similar between control M ϕ and co-cultured cells (M ϕ /MT2). Furthermore, glial cell death or a reduction in cellular metabolism was not detectable after culturing with the co-culture supernatant for 24h. Thus, our results suggested that contact between M ϕ and HTLV-1-infected cells promotes their activation and the release of WARS, but not of CXCL10. Further experiments are required to determine if WARS presence is associated with alterations in central nervous system cells. Keywords: HTLV-1;Macrophages;WARS.

2802 - Category: CELLULAR IMMUNOLOGY (CE)

IL-27 Co-expression Enhances CAR-NK Cell Effector Functions, and Modulates Immune Checkpoint DynamicsBIGGI, A.F.B.¹; SILVESTRE, R.N.²; TIRAPELLE, M.C.²; DOS SANTOS, M.H.²; EBRAHIMABADI, S.²; FERREIRA, C.M.G.²; SCHIMIDT, D.²; JANUARIO, M.E.S.²; CALADO, R.T.²; CASTRO, V.P.E.². 1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. FUNDAÇÃO HEMOCENTRO DE RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL.

CAR-NK cells are a promising tool in cancer immunotherapy, offering strong cytotoxicity and safer profile. Advancing these therapies through fourth-generation CAR constructs, co-expressing immunoregulatory cytokines, may further improve NK cell functions. IL-27 is a cytokine that modulates innate immunity and enhances NK cell activation, proliferation, and cytotoxic responses. We previously showed IL-27 enhances NK-92 functions through MAPK signaling, and here we extend these findings to primary CAR-NK cells targeting CD19. NK cells were isolated from leukoreduction chambers by Ficoll density gradient and expanded in AIM-V medium supplemented with 5% AB plasma and 200 IU/mL IL-2. Cells were transduced with a BaEV-pseudotyped lentivirus encoding either a CD19 CAR alone or a CD19 CAR co-expressing IL-27. Transduction efficiency for both constructs reach approximately 20%, as determined by CAR expression in flow cytometry. IL-27 CAR-NK cells demonstrated significantly increased cytotoxicity (P=0.01) and degranulation (CD107a⁺ frequency and MFI, P=0.01) in 4h co-culture with Raji and Namalwa CD19⁺ target cells at a 1:3 E:T ratio. Metabolic analysis revealed increased ATP production via glycolysis (P=0.001), suggesting enhanced metabolic fitness. Importantly, in 12h co-culture assays (1:2 E:T with Raji cells), IL-27 CAR-NK cells showed downregulation of the activating receptor NKp44 (P=0.001) and upregulation of the inhibitory receptor LAG-3 (P=0.001). Despite this, IL-27 CAR-NK cells maintained superior effector function, suggesting that enhanced glycolytic output may support early cytotoxic activity before checkpoint-mediated suppression becomes limiting. Together, these results highlight IL-27 as a promising cytokine for next-generation CAR-NK cells, with potential to overcome immune exhaustion through metabolic and signaling advantages. Research supported by FAPESP process 2023/09711-9, 2020/07055-9, FINEP 01.24.0656.00 ref. 0238-2024, 402853/2021-0. Keywords: Immunotherapy;CAR-NK;IL-27.

2813 - Category: CELLULAR IMMUNOLOGY (CE)

Maternal obesity promotes alterations in myeloid cells in the lungs of offspring in a murine model during early life stages

ALVES, D.C.D.A.; SILVA, R.S.F.; DE SOUZA, V.P.; MENEGATI, L.M.; DURAO, C.V.; DA SILVA, A.B.R.; IZOLAN, L.B.; DE OLIVEIRA, E.E.; SILVA, F.M.D.C.E.. UERJ, RIO DE JANEIRO - RJ - BRASIL.

Maternal obesity is associated with alterations in the offspring's immune system development, potentially compromising immune responses and increasing the risk of respiratory diseases (Mancuso, 2018). This study investigated the impact of maternal obesity on the composition and functionality of macrophages subtypes and dendritic cells in the lung of offspring, in a murine model. Female BALB/c mice were divided into two groups: Control (standard diet with 10% fat) and Obese (high-fat diet with 60% fat) for 10 weeks prior to mating. Offspring were evaluated at days -1 (gestational), 1, 3, 7, and 24 post-birth. Lungs were collected for histological analyses, flow cytometry, and cytokine quantification. No significant differences were observed in the number of alveolar (CD11c⁺ SIGLEC-F⁺) and interstitial (CD11b⁺ CD11c⁺ CD64⁺) macrophages. However, offspring of obese mothers showed a reduction in the frequency of dendritic cells (CD11c⁺ CD11b⁺ CD64⁺), along with decreased expression of TLR-4, TLR-2, MHC II, co-stimulatory molecule CD86, and phagocytic receptor CD64. Additionally, reduced levels of IL-33 were detected at all evaluated time points and of TSLP during the prenatal period. Histological analysis revealed a decreased number of eosinophils in lung tissue between days 3 and 7 of life. These results suggest that maternal obesity is associated with alterations in myeloid cells in the offspring's lung, potentially increasing susceptibility to respiratory diseases during early life stages. I gratefully acknowledge the funding agencies essential for the development of this research: CAPES, CNPq, and FAPERJ.

Keywords: Obesity;Offspring;Lung.

2817 - Category: CELLULAR IMMUNOLOGY (CE)

Effects of inulin, butyrate, and GPR43 receptor signaling on experimental acute psoriasis.

GASPAR, V.C.; SAGIORATO, R.N.; ALBIERO, I.; RODRIGUES, H.G.. UNICAMP, LIMEIRA - SP - BRASIL.

Introduction: Psoriasis is a chronic disease characterized by T cell activation, release of inflammatory mediators, and keratinocyte hyperproliferation. Inulin, a fiber fermentable by the microbiota, generates short-chain fatty acids (SCFAs), such as butyrate, with potential immunoregulatory effects, although its effects on psoriasis are poorly understood. In this study, our objective was to investigate the effects of supplementation with a high-fiber diet (10% inulin) and oral administration of butyrate in an acute psoriasis model. **Methods:** C57BL/6 mice were divided into control, psoriasis (IMQ), and psoriasis treated with inulin (IMQinu) or butyrate (IMQbut) groups. We used GPR43^{-/-}, as well as, S100A8cre⁺FFAR2^{fl/fl} mice to evaluate the action of butyrate in the absence of the GPR43 receptor and to investigate the effects mediated by neutrophils, respectively. Psoriasis was induced with topical application of 5% imiquimod (IMQ) for 5 days. Skin thickness, histological analysis, gene expression, and immunophenotyping of skin were evaluated. **Results:** The application of IMQ induced psoriatic inflammation, with increased skin/epidermis thickness and desquamation. Inulin supplementation reduced skin thickness, as well as epidermal area and thickness, similar to the results seen with butyrate. Butyrate increased the S100a8 gene expression, as well as, the percentage of macrophages MHC II^{low} and neutrophils. In GPR43^{-/-} mice, the beneficial effects of butyrate were abolished. S100A8cre⁺FFAR2^{fl/fl} mice showed an inflammatory response to induced psoriasis similar to that of wild-type mice. **Conclusion:** Our results confirm the beneficial effects of oral butyrate treatment during psoriasis. We also demonstrated the essentiality of GPR43 receptor to these effects. However, more experiments are necessary to unveil the molecular mechanisms. **Financial Support:** FAPESP and PRP/UNICAMP. This study was also financed by CNPq and CAPES: finance code 001. Keywords: Psoriasis;Butyrate;GPR43.

2829 - Category: CELLULAR IMMUNOLOGY (CE)

Depot-specific Adipose Tissue Secretomes from Lean and Obese Mice Differentially Modulate Macrophage Phenotypes

BARBOSA, M.¹; ANGELIM, M.K.S.C.²; CASTELUCCI, B.G.¹; SANTOS, W.G.S.¹; DOS SANTOS, T.P.¹; MARTINS, A.J.E.¹; TRIOZZI, E.¹; DA SILVA, G.R.¹; VIEIRA, P.M.M.D.M.¹. 1. UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS - SP - BRASIL; 2. SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE, ST. LOUIS, MO - ESTADOS UNIDOS DA AMERICA.

Obesity is characterized by the excessive accumulation of body fat. In addition to energy storage, adipose-tissue (AT) function as an endocrine organ, releasing bioactive molecules in its secretome, varying depending on the type and location of AT, influencing macrophages (MØ) phenotype. AT conditioned-medium (AT-CM) from perigonadal AT (PgAT) enhances lipid droplet formation and disrupts autophagy in MØ. The impact of other fat depots in MØ phenotype remains unclear. We aim to explore how CM from distinct AT depots from lean and obese mice modulates MØ phenotype. Mice were fed a chow (CD) or a high-fat-diet (HFD) for 12 weeks. Explants from subcutaneous (ScAT), PgAT, omental (OAT), perirenal (PrAT), and brown (BAT) AT were used to obtain AT-CMs. MØs were cultured for 6h and 24h with AT-CMs. We found that MØ treated with AT-CM are distributed along a spectrum of phenotypes. MØ treated for 6h with ScAT-CM from CD-fed mice expressed increased levels of Mgl1, Arg1 and Mrc1. PgAT-CM from HFD-fed mice led to increased expression of Tnf- α , Il-6, Il-10 and Arg1. CM derived from OAT of HFD-fed mice enhanced the expression of Itgax, Tnf- α Il-6 and Il-10 and decreased Mgl1 and Arg1 expression. PrAT-CM from HFD-fed mice increased expression the of Tnf- α , IL-1 β , Itgax, IL-6, Mrc1 and Mgl1and reduced Arg1. BAT-CM from HFD-fed mice selectively induced Il-6 expression. CMs from PgAT, OAT, and PrAT of HFD-fed mice stimulated TNF- α secretion after 24h. These findings deepen our understanding of MØ phenotypic modulation by adipose depots across the body. Keywords: Macrophages;Secretome;Adipose Tissue.

2853 - Category: CELLULAR IMMUNOLOGY (CE)

Pleural Cavity Macrophages Protect against Pulmonary Fibrosis

CASTANHEIRA, F.V.E.S.¹; DAVID, B.A.²; KONT, K.²; SALM, L.²; ZHAN, W.²; DENISET, J.²; THORNTON, C.²; KUBES, P.². 1. UNIFESP, SÃO PAULO - SP - BRASIL; 2. UNIVERSITY OF CALGARY, CALGARY - CANADA.

The pleural cavity, traditionally viewed as a mechanical aid in respiration, harbors a dense population of cavity-resident macrophages, predominantly expressing Gata-6. Despite their proximity to the lung surface, the role of pleural macrophages (PMs) in lung disease has been largely unexplored. In this study, we used a bleomycin-induced pulmonary fibrosis model and mice with conditional deletion of Gata-6 in myeloid cells (Lyz2Cre;Gata6^{fl/fl}) to investigate the immunological contribution of PMs. We observed that while all wild-type mice survived bleomycin-induced fibrosis, the absence of PMs led to decreased survival. In addition, depletion of pleural macrophages led to enhanced lung inflammation, characterized by elevated recruitment of eosinophils and neutrophils to both the pleural space and parenchyma, exacerbating fibrotic responses. Interestingly, PMs did not infiltrate lung tissue but remained within the cavity, adhering to the mesothelial surface and likely modulating local immune responses via paracrine mechanisms. The protective effect was intrinsic to the GATA6⁺ macrophages as replacement of these cells rescued the protective phenotype. Although VEGF production by PMs has some protective impact, our results suggest that other mediators and cell-cell interactions are critical for their immunoregulatory function. Furthermore, human PMs displayed similar phenotypic features, supporting evolutionary conservation. Altogether, this work identifies pleural macrophages as key non-invasive regulators of lung inflammation, with therapeutic potential in fibrotic lung diseases. Keywords: Pleural Macrophages;Pulmonary Fibrosis ;Inflammation.

2857 - Category: CELLULAR IMMUNOLOGY (CE)

Evaluation of the effect of Bacillus Calmette-Guérin on the process of senescence of T lymphocytes

MOREIRA, B.L.C.¹; PERUSSOLO, M.C.¹; NISIMURA, L.M.¹; DALLAZUANA, V.¹; NASCIMENTO, C.D.M.C.¹; RIBEIRO, C.L.G.¹; BATISTA, M.N.²; WOWK, P.F.¹; MOSIMANN, A.L.P.³; BORDIGNON, J.³. 1. ICC - FIOCRUZ/PR, CURITIBA - PR - BRASIL; 2. THE ROCKEFELLER UNIVERSITY, NEW YORK - ESTADOS UNIDOS DA AMERICA; 3. ICC - FIOCRUZ/PR, CURITIBA - PR - BRASIL.

The BCG vaccine has been used for over a century to prevent severe forms of tuberculosis in children and later as an adjunct treatment for bladder cancer. Recently, its immunomodulatory function has been more broadly analyzed, as studies have shown its protective potential for other unrelated diseases, such as viral respiratory infections. Of note, researches focused on immune system aging, known as immunosenescence, have reported BCG's ability to mitigate this process by training the innate immune system to become more efficient in combating unassociated infections. We propose to evaluate the effect of BCG vaccination in C57BL/6 mice as they age, analyzing the cellular and functional profiles of their immune system. Mice (8 weeks) were divided in five groups (I – 3x PBS; II – PBS/PBS/BCG; III – 3x BCG; IV – BCG/BCG/PBS; V – BCG/PBS/PBS) and immunized three times intravenously, every six months, with different combinations of PBS or BCG vaccine. Thirty days after each immunization, a portion of mice from each group was euthanized and spleen and sera were collected. Flow cytometry analysis was done to evaluate the distribution of major immune cell compartments, focusing on T cell subsetting (regulatory, follicular, naïve/memory) and functional profiling (activation, exhaustion, senescence, cytokines production). After the first immunization, partial results indicate that the ratio CD4/CD8 T cells in spleen had non-significant increase in BCG groups, also there is a considerable reduction of follicular, exhausted, regulatory and senescent T cells in this group. However, production of cytokines and activation of said cells remained relatively the same. At this point, the remaining mice received the second immunization, a portion was euthanized and the data collected is being analyzed. As mice age, we expect to see more differences between groups, especially with BCG having more prominent effects regarding exhaustion, regulatory and senescence of T lymphocytes. Keywords: BCG;immunosenescence;T lymphocytes.

2866 - Category: CELLULAR IMMUNOLOGY (CE)

Central Obesity Alters T-Cell Function and Metabolism: Protective Role of lifelong Physical Activity

OLEAN-OLIVEIRA, T.¹; SILVA, B.S.D.A.¹; SARDELI, A.V.²; ANTUNES, B.M.¹; DE OLIVEIRA, P.E.M.¹; DUGGAL, N.²; TEIXEIRA, J.³; COELHO-E-SILVA, M.J.⁴; OLIVEIRA, P.J.³; LIRA, F.S.¹. 1. EXERCISE AND IMMUNOMETABOLISM RESEARCH GROUP, POST GRADUATION PROGRAM IN MOVEMENT SCIENCES - UNIVERSIDADE ESTADUAL PAULISTA (UNESP), PRESIDENTE PRUDENTE - SP - BRASIL; 2. INSTITUTE OF INFLAMMATION AND AGEING, UNIVERSITY OF BIRMINGHAM, BIRMINGHAM - REINO UNIDO; 3. UNIV COIMBRA, CENTER FOR INNOVATIVE BIOMEDICINE AND BIOTECHNOLOGY (CIBB), COIMBRA - PORTUGAL; 4. 4UNIVERSITY OF COIMBRA, CIPER, FACULTY OF SPORT SCIENCES AND PHYSICAL EDUCATION, COIMBRA - PORTUGAL.

Aging, central obesity, and physical inactivity lead to chronic low-grade inflammation which impairs immune function and increases the risk of chronic diseases. Regular physical exercise reduces adiposity and induces positive immunometabolic adaptations. However, the impact of lifelong physical activity (PA) on CD4⁺/CD8⁺ T cell immunometabolism remains unclear. This study investigated the impact of lifelong physical activity on the function and metabolism of CD4⁺ and CD8⁺ T cells in adults with and without central obesity. Eighteen adults (18–77 years) were stratified using central obesity (waist circumference-CO) into High-CO (≥ 94 cm; 101.1 ± 4.1 cm; 45 ± 20 years; $n=7$) and Low-CO (< 94 cm; 78.5 ± 7.4 cm; 39 ± 21 years; $n=11$) groups. All participants completed validated PA levels questionnaires. After overnight fasting, blood samples were collected to assess 1) CD4⁺/CD8⁺ T-cell cytokine expression in the presence or absence of phorbol 12-myristate 13-acetate [50 ng/mL] plus ionomycin [1 μ g/mL] (4 hours, 37°C, 5% CO₂), 2) the glycolytic and mitochondrial activities of CD4⁺/CD8⁺ T cells were evaluated using SCENITH and 3) CD4⁺ metabolism, oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using Seahorse XF96 under same stimulated conditions. TNF- α and IFN- γ production, glycolysis, and OCR were similar between groups. Under resting conditions, higher CO inversely correlated with CD4⁺ mitochondrial activity after adjusting for age ($r = -0.65$; $p=0.01$), but not after PA adjustment ($r = -0.57$; $p=0.07$). Upon stimulation, CO was also inversely correlated with CD4⁺ ECAR ($r = -0.59$; $p=0.02$). The High-CO group showed a trend toward lower IL-6 and significantly higher IL-4 ($p = 0.02$) and IL-10 ($p = 0.03$) expression in CD4⁺ T cells (IL-6: $p = 0.067$), and higher IL-17 production in CD8⁺ T cells ($p = 0.01$) compared to Low-CO. Finally, while central obesity impaired immune metabolism and cytokine regulation, lifelong physical activity partially preserved immune function. Keywords: T-cell immunometabolism;Central obesity;Exercise.

2878 - Category: CELLULAR IMMUNOLOGY (CE)

Low protein diet effect on lung immune homeostasis and inflammation

DE ABREU, A.M.; ALVES, C.B.; DA CONCEIÇÃO, L.B.; DE ASSIS, J.B.; DA SILVA, G.W.; CARDOSO, A.; DE ARAUJO, M.V.; RODRIGUES, G.M.B.; GONÇALVES, L.M.; OLIVEIRA, B.D.C.; COELHO, M.P.S.; CAMARGO, L.; AYUPE, M.C.; DA FONSECA, D.M.. LABORATORY OF MUCOSAL IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES - USP / NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY FOR RESEARCH IN INFECTIOUS AND CHRONIC DISEASES OF THE MUCOSA AND SKIN, SÃO PAULO - SP - BRASIL.

Mucosal surfaces are continuously exposed to antigens from the external environment. Because of this, the immune system associated with organs, such as the intestine and lungs, requires effective barrier mechanisms to prevent excessive or unnecessary immune responses while maintaining protection against infections. Despite their distinct physiological roles, the intestine and lungs are immunologically connected through the gut–lung axis. Factors such as dietary changes can alter the immune profile and the gut microbiota composition, which may in turn impact pulmonary homeostasis. Previous findings from our group showed that a low-protein diet shifts the immune tone of the proximal small intestine toward a Th2-driven and regulatory profile. However, the impact of this diet on pulmonary immunity remains unclear. We hypothesized that a low-protein diet would alter the pulmonary immune environment and local microbiota, promoting inflammatory responses and increasing susceptibility to diseases such as allergic asthma. To test this, mice were fed a low-protein diet for six weeks after weaning, and asthma was induced using a papain/OVA-based protocol. Our results show that the dietary change (even without the allergy induction) was sufficient to modulate the pulmonary environment toward a Th2 profile, marked by an increased frequency of eosinophils. However, our initial hypothesis was not confirmed, as no exacerbation of allergic asthma was observed in mice fed the low-protein diet. Additionally, diet consumption altered the cultivable lung microbiota, favoring a species capable of growing on Bile Esculin Agar, but without esculin hydrolysis. As next steps, we will replicate the model and perform detailed cultivable microbiota characterization and sequencing. Furthermore, we will evaluate the role of lung microbiota, altered by the low-protein diet, in lung immunity. Keywords: Low-protein diet; Allergic asthma; Gut-lung axis.

2879 - Category: CELLULAR IMMUNOLOGY (CE)

Displacement of Peritoneal B-1 Lymphocytes After Adoptive Transfer in Diabetic Mice: Preliminary Observations and Open Questions

CORONA, L.M.¹; PHELIPPE, N.E.¹; DA SILVA, A.P.S.¹; PINHEIRO, C.O.D.D.¹; CURI, R.²; VESSONI, S.C.S.³; LALLO, M.A.¹; ALVARES-SARAIVA, A.M.¹. 1. UNIP, SÃO PAULO - SP - BRASIL; 2. UNICSUL, SÃO PAULO - SP - BRASIL; 3. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

B-1 lymphocytes are recognized for their multifunctionality and their ability to differentiate into other cell types. Previous studies, including the adoptive transfer of these cells, have demonstrated resistance to streptozotocin-induced diabetes in XID mice, which are deficient in B-1 lymphocytes. Furthermore, these cells have shown the capacity to produce insulin and regulate glycemia in animal models. Additionally, *in vitro* metabolic reprogramming has proven effective in enhancing the insulin-producing capacity of these cells. The present study aimed to track metabolically reprogrammed B-1 lymphocytes for insulin production following adoptive transfer into diabetic mice. Accordingly, B-1 lymphocytes obtained from wild-type mice were chemically reprogrammed and subsequently transferred into XID mice previously treated with streptozotocin to induce diabetes. Nine days post-transfer, one of the three animals exhibited a glycemic level of 211 mg/dL and did not develop diabetes. Phenotypic analysis indicated the absence of B-1 lymphocytes in the peritoneal cavity of the healthy animal, whereas in the diabetic animals, the population ranged from 5% to 18%. In the spleen, B-1 lymphocytes represented approximately 2%, and in the liver, about 3% in all animals. Pancreatic histopathological analysis revealed few or no preserved islets in the diabetic animals, whereas the normoglycemic animal presented good vascularization, large and regular islets, and numerous vacuoles. These data suggest that the transfer of insulin-producing B-1 lymphocytes can promote normoglycemia in murine models of diabetes. Further investigation is ongoing to determine whether B-1 lymphocytes infiltrate the pancreas and which mechanisms govern the migration and/or persistence of these cells in the peritoneal cavity. Keywords: B-1 lymphocytes; Diabetes; Metabolic reprogramming.

2887 - Category: CELLULAR IMMUNOLOGY (CE)

Clinical, Laboratory, and Ancestry Characterization of a Cohort of Young Adult Brazilians with Severe COVID-19.

DE JESUS, T.S.; LESSA, A.C.M.; DE SOUSA, E.S.C.; PEDRO, R.B.D.S.; DE SOUZA, A.C.L.; NOVAES, S.D.; DALTRO, J.D.P.; OLIVEIRA, P.R.S.; AMARAL, E.H.B.; SANTOS, A.O.M.; MARINHO, A.I.. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL.

The coronavirus disease caused by SARS-CoV-2 has been the subject of intense scientific research aimed at identifying factors that favor the development of severe forms of the disease. According to the World Health Organization, by the end of 2024, more than 700 million individuals may have contracted severe infections, and approximately 7.1 million deaths will have been associated with COVID-19. Risk factors, including age, sex, and comorbidities, influence the severity of the disease. However, some young, previously healthy individuals develop critical forms of the disease, suggesting genetic predispositions. This study aims to characterize the clinical, laboratory, sociodemographic, and genomic ancestry of previously healthy patients who were hospitalized with severe COVID-19. This descriptive cross-sectional study collected data from the medical records of 159 patients (aged 18-60 years) recruited from seven hospitals in Brazil between August 2020 and August 2021. Descriptive analyses were performed using GraphPad Prism and RStudio. Serious complications, including Acute Respiratory Distress Syndrome, were observed in 32.1% of cases. Laboratory tests revealed significant increases in neutrophil count (Median: 8307, IQR: 8065 - 8729.5), AST (Aspartate Aminotransferase) levels, (Median: 58.2, IQR: 53 - 60.75), ALT (Alanine Aminotransferase) levels, (Median: 68.45, IQR: 48.5 - 73.5) and CRP (C-Reactive Protein) levels, (Median: 8.2, IQR: 8.2 - 126.5). Genomic ancestry analysis revealed that most patients have admixed genomic ancestry, with contributions from the superpopulations of Europe (EUR), Median: 0.61, America (AMR), Median: 0.10, and Africa (AFR), Median: 0.23. Thus, studies that integrate genetic, sociodemographic, and clinical-laboratory data are essential not only to confirm or contrast findings from the beginning of the pandemic but also to comprehensively describe severe cases of COVID-19, contributing to the understanding of its multiple determinants. Keywords: Severe COVID-19; Genomic ancestry; Risk factors.

2899 - Category: CELLULAR IMMUNOLOGY (CE)

Immunophenotyping of T Lymphocytes in Sepsis: Characterization of Activation, Exhaustion, Anergy, and Senescence.

SILVA, J.G.D.¹; SOUSA, M.B.²; EBURNEO, G.S.¹; SANTOS, S.S.¹; LEITE, G.G.F.¹; SALOMÃO, R.¹. 1. UNIVERSIDADE FEDERAL DE SÃO PAULO (UNIFESP), SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Introduction: Septic patients present with lymphopenia; furthermore, the remaining lymphocytes exhibit signs of senescence and exhaustion, resulting in a compromised immune response.

Objective: To characterize the states of activation, senescence, exhaustion, and anergy in CD4⁺ and CD8⁺ T lymphocyte populations in septic patients.

Methods: Sixty patients with sepsis were selected, with samples collected at ICU admission (D0) and seven days after the first collection (D7), along with 20 healthy volunteers as controls (CT). Ten different antibodies related to the functional states of interest were used and analyzed by flow cytometry. The analyses were performed using FlowJo, and the data were processed in R software.

Results: Both CD4⁺ and CD8⁺ T lymphocytes showed increased expression of anergy (CTLA-4) and activation (HLA-DR, CD38) markers in patients compared to controls; CD4⁺ T lymphocytes also showed higher expression of the exhaustion marker (PD-1). An increase in anergic naïve CD4⁺ and CD8⁺ T lymphocytes (CTLA-4) was observed in patients.

Through a clustering analysis (t-SNE), 11 distinct clusters were identified. Some showed the concomitance of more than two functional profiles. In the comparison between the cell populations of each cluster, cluster 3 (activated naïve CD4 T lymphocytes) and cluster 9 (activated effector memory RA CD8 T lymphocytes) showed lower frequency in patients. In contrast, cluster 5 (exhausted and activated central memory CD4 T lymphocytes) showed an increased frequency in patients.

Conclusion: The lymphocytes of septic patients show higher expression of activation markers, concomitant with exhaustion and anergy markers, which demonstrates a dysfunctional state. The alterations were more evident in CD4 T lymphocytes than in CD8. The tSNE reinforces some data from the comparison between controls and patients, in addition to showing that it is possible for lymphocytes to simultaneously present signs of activation, exhaustion, and senescence. Keywords: Sepsis; Immunophenotyping; Lymphocytes.

2912 - Category: CELLULAR IMMUNOLOGY (CE)

Inhibitory Role of GXM and GXMGal purified from *Cryptococcus neoformans* on Phagocytosis and Microbicidal Activity in Feline Macrophage cell line Fcwf-4

DOS SANTOS, R.Q.¹; ABRANTES, L.S.¹; DA SILVA JUNIOR, E.B.²; MOREIRA, F.D.P.P.¹; VELEZ, A.S.M.M.³; MEYRELLES, M.S.M.¹; PREVIATO, L.M.²; PREVIATO, J.O.²; DA SILVA, L.H.P.¹; FREIRE-DE-LIMA, C.G.²; DECOTE-RICARDO, D.¹. 1. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO - INSTITUTO DE VETERINÁRIA, SEROPÉDICA - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - INSTITUTO CARLOS CHAGAS FILHO, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO - INSTITUTO DE QUÍMICA, SEROPÉDICA - RJ - BRASIL.

Cryptococcus neoformans and *Cryptococcus gattii* are the species responsible for cryptococcosis, a multisystemic disease and the most common mycosis in cats. The infection initially targets the respiratory system and may disseminate to the central nervous system. The main virulence factors of this fungus is its capsule, composed of polysaccharides glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal). These polysaccharides possess immunomodulatory properties, and one of their primary cellular targets is macrophages. Macrophages are part of the first line of defense, playing a key role in antifungal responses, essential in innate immunity, and crucial for the development of adaptive immunity. There is no information on the interaction between *C. neoformans* and feline immune cells, as well as the modulatory effects of its capsular components. This study aimed to evaluate whether *C. neoformans* polysaccharides are able of modulating the microbicidal activity of feline macrophage Fcwf-4. Feline macrophages were incubated with opsonized or non-opsonized *C. neoformans* yeasts to assess their phagocytic activity. The effects of polysaccharides on macrophage phagocytosis were also evaluated. Additionally, we assessed the impact of these polysaccharides on the production of reactive oxygen species (ROS) by macrophages treated with GXM and GXMGal. The results demonstrated that feline macrophages are capable of phagocytosing *C. neoformans* yeasts, and this activity is enhanced by opsonization. GXM and GXMGal inhibited phagocytosis without exerting cytotoxic effects on feline macrophages. Furthermore, a reduction in ROS production was observed. Collectively, these findings suggest that feline Fcwf-4 macrophages can phagocytose *C. neoformans* yeasts, and that the capsular polysaccharides GXM and GXMGal exert inhibitory effects on both phagocytic activity and ROS production, indicating a suppressive effect on the microbicidal function of these macrophages. Keywords: *Cryptococcus neoformans*; Feline macrophage; Capsular polysaccharides.

2917 - Category: CELLULAR IMMUNOLOGY (CE)

INCREASED SUSCEPTIBILITY TO ACUTE KIDNEY DISEASE AFTER EXPERIMENTAL CHRONIC INTESTINAL INFLAMMATION

ANDRADE-OLIVEIRA, V.¹; NERY-NETO, J.A.D.O.²; VIEIRA, R.D.S.²; YARIWAKE, V.Y.²; YAMADA, S.M.²; DA SILVA, E.M.³; SOUZA, C.S.²; DA SILVA, J.V.H.²; CIPELLI, M.²; OLIVEIRA, S.D.S.⁴; DA SILVEIRA, A.G.²; PADOVANI, B.N.²; QUADROS-PEREIRA, L.¹; DORETTO-SILVA, L.¹; CAMARA, N.O.S.⁵. 1. CENTER FOR NATURAL AND HUMAN SCIENCES – FEDERAL UNIVERSITY OF ABC, SANTO ANDRÉ - SP - BRASIL; 2. DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. DIVISION OF NEPHROLOGY, SCHOOL OF MEDICINE, FEDERAL UNIVERSITY OF SÃO PAULO, SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 5. DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL.

Introduction: Renal manifestations in patients with ulcerative colitis and Crohn's disease suggest a connection between inflammatory bowel diseases (IBD) and kidney dysfunction. However, the mechanisms underlying this remain unclear. Enteroendocrine cells (EECs), distributed throughout the gastrointestinal tract, secrete hormones such as GIP, GLP-1, and GLP-2, incretins that have shown renoprotective effects. Nevertheless, whether the EECs and the incretins play any role in the increase of renal disorders in Chronic Intestinal Inflammation (CII) is not known. **Objective:** This study aims to evaluate the influence of CII in an Acute Kidney Injury (AKI) model. **Methods:** C57BL/6 mice were subjected to three cycles (5 days each) of 2% dextran sulfate sodium (DSS) in water, with a 4-day interval between cycles. The Disease Activity Index (DAI) was determined based on weight loss, stool consistency, and rectal bleeding. The predisposition to renal injury was evaluated using an ischemia-reperfusion (I/R) Injury model for 15 minutes. The frequencies of immune cells were assessed using flow cytometry. **Results:** Although renal function was not directly affected by colitis, increased expression of KIM-1, an early marker of renal injury, was observed, in addition to anemia and increased activation of the NF- κ B pathway in the kidney, without changes in the profile of cytokines such as IL-1 β , IL-6, IL-10, TGF- β , and TNF- α . CII animals exhibited greater susceptibility to ischemic AKI, evidenced by increased recruitment of renal monocytes after I/R. In addition, a reduction in CEEs was observed in the colon of animals exposed to DSS. **Conclusion:** CII leads to elevated kidney injury molecular markers, along with the recruitment of monocytes and NF- κ B activation, culminating in the susceptibility to AKI. **Financial Support:** FAPESP (2022/08362-8; 2019/14755-0). Keywords: Acute kidney Injury; Colitis; Enteroendocrine cells.

2927 - Category: CELLULAR IMMUNOLOGY (CE)

Role of NFAT1 transcription factor in the generation and function of Memory-like CD8⁺ T lymphocytes in young and aged mice

BARBOZA, B.A.; VIEIRA, B.L.M.D.O.; PORTO, H.F.B.; FERREIRA, L.V.L.; FREIRE, L.L.; POLIDO, V.P.; SEBBA, M.E.C.; SILVA, K.L.; VIOLA, J.P.D.B.. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

Memory-like cells, also known as Memory-phenotype, are antigen-inexperienced memory T cells present within hosts that have not been exposed to foreign antigen(s), such as non-immunized/unprimed mice. It is described that this population increases with aging. Knowing that NFAT1 transcription factor has an important role in "classic" CD8 T lymphocytes, we questioned its part in the generation and function of Memory-like CD8 T cells during aging. When analyzing peripheral immune tissues (peripheral lymph nodes and spleen) from non-immunized mice by Flow Cytometry, we observed that young and aged mice lacking NFAT1 (NFAT1^{-/-}) presented a higher frequency of cells expressing high levels of CD44 compared with the control group (C57BL/6). As CD44 is a marker of activation, these results suggest an increase in activated/memory cells in the knockout mice (NFAT1^{-/-}). However, most of this population did not express CD49d and expressed high levels of CD62L and CD127, indicating that these are Memory-like cells. We also noted that this group of cells increased with age when we compared Middle-Aged (13-16 months) and Old (22-23 months) mice with the younger ones (2-3 months). We are still planning on analyzing additional markers, like NKG2D and T-bet, so we can better classify these cells. We also going to study the expression of some transcription factors in the Thymus, like PLZF and Eomes, to understand the generation of this group of cells. Furthermore, we intend to analyze functional properties *in vitro*, like IFN-gamma and Granzyme B production, proliferation, and cytotoxic capacity. **Financial support:** CNPq and FAPERJ. **Keywords:** Memory-like;NFAT1;Aging.

2954 - Category: CELLULAR IMMUNOLOGY (CE)

Serum Amyloid A (SAA) in Chronic Wounds: Inflammation and Pain

SALGADO, B.F.¹; DIAS, B.B.¹; FELIPE, M.G.¹; GONZALEZ, C.S.²; CARNEIRO, P.²; DE GOUVEIA, V.L.C.²; DERMARGOS, A.¹; DERMARGOS, E.H.¹. 1. UNIVERSIDADE CRUZEIRO DO SUL, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Serum Amyloid A (SAA) plays a key role in the acute-phase response following injury. This protein is involved in tissue remodeling by promoting inflammatory responses essential for wound healing. SAA fragments may contribute to amyloid formation, which can impair normal tissue repair processes. Its interaction with receptors influences local tissue changes and modulates healing, particularly in inflammatory and fibrotic conditions. This study aimed to analyze and correlate SAA concentrations found in chronic wounds with wound area, pain intensity reported by patients, and the systemic inflammatory state. Patients with ulcerative wounds treated at the stomatherapy unit (protocol numbers 3.733.692 / 5.056.116 / 5.189.744) were evaluated in two to three sessions. After obtaining informed consent, venous blood and wound exudate samples were collected to quantify SAA. In addition, clinical evaluations were conducted, including analysis of wound location and measurement, edema, and pain, along with photographic records and documentation of wound characteristics. No significant correlations were found between pain scores and SAA levels in serum or exudate. However, a positive correlation was observed between wound area and SAA concentration in the exudate, suggesting that larger wounds present higher local levels of this protein ($r^2 = 0,1101$; $p = <0,0001$). **Keywords:** Inflammation;Pain;Serum Amyloid A.

2967 - Category: CELLULAR IMMUNOLOGY (CE)

Pulmonary infection by *Streptococcus pneumoniae* modulates cellular infiltration in the intestinal mucosal compartments

DE ARAUJO, M.V.¹; CAMARGO, L.¹; MACHADO, L.F.²; DA SILVA, G.W.¹; OLIVEIRA, B.D.C.¹; GONÇALVES, L.M.¹; RODRIGUES, G.M.B.¹; DE ASSIS, J.B.¹; DA CONCEIÇÃO, L.B.¹; ALVES, C.B.¹; DE ABREU, A.M.¹; COELHO, M.P.S.¹; CARDOSO, A.¹; DA FONSECA, D.M.¹. 1. LABORATORY OF MUCOSAL IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES - USP / NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY FOR RESEARCH IN INFECTIOUS AND CHRONIC DISEASES OF THE MUCOSA AND SKIN, SÃO PAULO - SP - BRASIL; 2. FEDERAL UNIVERSITY OF JUIZ DE FORA, SÃO PAULO - SP - BRASIL.

The mucosal tissue, which covers surfaces such as the lungs, intestines, oropharynx, and urogenital tract, constitutes an essential immune barrier against pathogens and is also a functional link between different tissues. The immune response in these tissues forms a primary response against pathogens. In addition to the classical mechanisms of immunity described for barrier tissues, it is also known that these tissues maintain intense communication. The gut-lung axis, for example, is defined by the cross-talk between the intestinal and pulmonary mucosa, mediated by cells of the immune system, microorganisms, microbial components and metabolites, chemical mediators, and other endogenous humoral regulators. Our study investigated how pulmonary infection by *Streptococcus pneumoniae* (Sp), an encapsulated Gram-positive pathogen associated with invasive diseases such as pneumonia, modulates the gut mucosal immune response and confers cross-protection against gastrointestinal infection by *Yersinia pseudotuberculosis* (Yp). In a murine model, we observed that mice previously infected with Sp presented a decrease in macrophage populations in the colon of these animals on the 3rd day after infection, which reestablished itself by the 7th day. In addition, we observed that the previous infection induced significant protection against Yp, evidenced by the absence of body weight loss and mesenteric lymphadenopathy. Immunophenotyping revealed an increase in IFN- γ -producing CD8+ and CD4+ lymphocytes in the lungs, indicating a local Th1 response. In addition, an accumulation of Th17 cells was detected in the mesenteric tissue, suggesting activation of the lung-gut axis. We hypothesize that pulmonary infection induces a state of trained immunity, with functional reprogramming of immune cells, particularly macrophages, promoting heterologous resistance. Keywords: *Streptococcus pneumoniae*;lung-gut axis;mesentery.

2971 - Category: CELLULAR IMMUNOLOGY (CE)

Evaluation of the Immune Response After Treatment of Experimental Envenomation by *Bothrops jararacussu* Using Polyclonal Serum and Camelid Nanobodies

SANTANA, H.M.; FARIAS, B.J.C.; SILVA, M.D.S.; DA SILVA, C.P.; EULÁLIO, M.D.M.C.; FERREIRA, A.A.F.E.; DOS REIS, V.P.; IKENOBUCHI, Y.J.; PALOSCHI, M.V.; DE LIMA, A.M.; SOARES, A.M.; PEREIRA, S.D.S.; SETÚBAL, S.D.S.; ZULIANI, J.P. FUNDAÇÃO OSWALDO CRUZ RONDÔNIA, PORTO VELHO - RO - BRASIL.

In Latin America, snakebite envenomation is both a neglected and occupational disease. Globally, over 420,000 cases occur annually, with around 20,000 deaths. In Brazil, most cases are caused by snakes from the *Bothrops* genus. While antivenoms can neutralize systemic effects, local symptoms like myonecrosis, dermonecrosis, pain, and edema often persist. A major contributor to these local effects is snake venom phospholipases A₂ (svPLA₂s), which cause significant cytotoxic and myotoxic damage. Camelid hyperimmune serum, particularly from *Lama glama*, contains unique heavy-chain antibodies (HcAbs), also called nanobodies (VHH), which are smaller, more stable, and less immunogenic than conventional antibodies. These properties make them promising therapeutic agents. In this study, *Lama glama* was immunized with whole venom and svPLA₂s from *Bothrops jararacussu* (vBj). The resulting antibodies (IgG1, IgG2, and IgG3) were purified, with yields of 4 mg/mL, 1 mg/mL, and 200 μ g/mL, respectively. To assess efficacy, Swiss mice were injected with 25 μ g of vBj and treated with 7 μ g of HcAbs. Blood analyses showed significant reductions in muscle damage (CK), cytotoxicity (LDH), and cardiac injury (CK-MB) compared to untreated mice. Acute liver injury (AST) was also reduced, while chronic injury marker (ALT) remained unchanged. Additionally, IL-1 β levels, linked to inflammasome activation, were significantly lowered in HcAb-treated animals. These findings demonstrate that HcAbs can effectively mitigate local tissue damage and inflammation in envenomated mice, supporting their potential as complementary therapies alongside traditional antivenoms in treating snakebite envenomation. Keywords: Snake Venoms;Camelids;Bothrops.

2972 - Category: CELLULAR IMMUNOLOGY (CE)

Immunomodulatory Effects of Benznidazole on Double Negative Effector Memory T Cells in Chronic Chagas Disease

KOH, C.C.¹; LUCINDA, P.P.D.¹; SILVA, S.D.A.¹; NUNES, M.D.C.P.¹; RIBEIRO, A.¹; BARROS, M.¹; NORONHA, A.²; GALVÃO, L.²; CÂMARA, A.C.²; BEDOR, D.C.G.³; SANTANA, D.P.³; TACONELLI, C.⁴; PADILHA, J.L.⁴; CARVALHO, A.T.⁵; GOLLOB, K.J.⁶; CAMPOS, L.⁷; SABINO, E.C.⁷; DUTRA, W.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE, NATAL - RN - BRASIL; 3. UNIVERSIDADE FEDERAL DE PERNAMBUCO, RECIFE - PE - BRASIL; 4. UNIVERSIDADE FEDERAL DO PARANÁ, CURITIBA - PR - BRASIL; 5. FIOCRUZ, BELO HORIZONTE - MG - BRASIL; 6. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 7. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Introduction: Chagas disease, caused by *Trypanosoma cruzi*, is a chronic infection with clinical forms ranging from indeterminate (IND) to cardiac (CCC) and/or digestive involvement. Benznidazole (BZ), the first-line therapy, has proven parasitological efficacy; however, its effects on human immune response remain poorly understood. Double negative (DN, CD4⁺CD8⁻) T-cells are a major source of inflammatory and anti-inflammatory cytokines in human Chagas disease. We hypothesized that BZ therapy modulates DN T-cell phenotype and function in association with treatment outcomes.

Methodology: PBMCs from 22 BZ-treated patients (IND = 13; CCC = 9) were collected before and six months post-treatment. DN T-cell phenotype and function were assessed by multiparametric flow cytometry (FACS Symphony A5), and parasitemia was evaluated by PCR for *T. cruzi* DNA. **Results:** Post-treatment, IND patients showed a significant increase in effector memory (EM) DN T cells, with a shift toward Th1-type polarization: increased expression of and CD183 (CXCR3), and decreased CD294 (CRTH2), suggesting functional reprogramming. In contrast, CCC patients showed reductions in CD183⁺ and CD336⁺ EM DN T cells, indicating limited or altered immune reactivity. Importantly, CD183 expression was elevated in patients with negative PCR results post-treatment, supporting its association with parasite control. **Conclusion:** BZ therapy induces functional and phenotypic reprogramming of EM DN T cells, particularly in IND patients, enhancing Th1-like responses and immune competence. These findings highlight the immunomodulatory effects of treatment and support **CD183 expression as a potential candidate marker of therapeutic response** in chronic Chagas disease. **Keywords:** Double-negative T cells; Chronic Chagas disease; Benznidazole immunomodulation.

2976 - Category: CELLULAR IMMUNOLOGY (CE)

SINGLE-CELL TRANSCRIPTOMIC PROFILING REVEALS INFLAMMATORY AND CYTOTOXIC SIGNATURES IN ANTIGEN-PRESENTING CELLS FROM PATIENTS WITH CHRONIC CHAGAS CARDIOMYOPATHY

FERNANDES, L.C.¹; SILVA, T.G.D.S.E.²; LUCINDA, P.P.D.¹; FIGUEIREDO, A.B.³; MORAIS, K.L.P.³; APOSTÓLICO, J.³; PANTALEÃO, A.⁴; MUTARELLI, A.⁴; SILVA, S.D.A.⁴; DE CARVALHO, A.T.⁵; NUNES, M.D.C.P.⁴; GOLLOB, K.J.³; DUTRA, W.¹. 1. LABORATORY OF CELL-CELL INTERACTIONS, DEPARTMENT OF MORPHOLOGY, INSTITUTE OF BIOLOGICAL SCIENCES, UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. DEPARTMENT OF IMMUNOLOGY, HARVARD UNIVERSITY, BOSTON - ESTADOS UNIDOS DA AMERICA; 3. CENTER FOR RESEARCH IN IMMUNO-ONCOLOGY, CRIO, HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 4. DEPARTMENT OF CLINICAL MEDICINE, SCHOOL OF MEDICINE, UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 5. INSTITUTO RENÉ RACHOU, FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Chagas disease, caused by *Trypanosoma cruzi*, significantly alters the immune response, especially in Chronic Chagas Cardiomyopathy (CCC), the severe, symptomatic form with high morbidity and mortality, compared to the asymptomatic indeterminate (IND) form. Myocardial damage in CCC is driven by parasite persistence and a pro-inflammatory profile mediated by inflammatory cytokines and cytotoxic molecules. Antigen-presenting cells (APCs), including monocytes and dendritic cells (DCs), are vital for mounting and maintaining immune responses through cytokine production and T-cell activation. Twenty-four IND and CCC patients were recruited at Hospital das Clínicas/UFMG for peripheral blood collection. Peripheral blood mononuclear cells (PBMCs) were isolated and subjected to single-cell RNA sequencing using the BD Rhapsody platform and an immune panel of 400 genes. Sequencing and statistical analysis were performed on NovaSeq™ 6000 and GraphPad Prism 10™. Analyzing the transcriptional changes in innate myeloid cells, we identified a population of dendritic cells and four distinct monocyte populations: classical, non-classical, intermediate, and classical pro-inflammatory. The monocyte subpopulations upregulated distinct chemotactic and immunoregulatory genes when comparing IND and CCC. Notably, there was an upregulation of the IFNG gene, not commonly observed in monocytes. Dendritic cells in the IND cohort were characterized by the upregulation of CD34, ICAM1 and T-cell chemotactic genes. In CCC, dendritic cells upregulated the expression of inflammatory transcriptional and chemotactic genes and, interestingly, cytotoxic genes, including GNLY, PRF1, GZMA, and GZMB. *In vitro* analysis of IFN-gamma and granzyme expression by monocytes from CCC patients confirmed the presence of these molecules, particularly after antigenic stimulation. These findings suggest a role for these atypical cell populations in the pathogenesis of CCC. **Keywords:** Chagas disease; Antigen-presenting cells (APCs); Immune signatures.

2982 - Category: CELLULAR IMMUNOLOGY (CE)

MECHANISMS MEDIATING THE PULMONARY NEUTROPHILIA INDUCED BY KETOGENIC DIET

CARDOSO, A.; RODRIGUES, G.M.B.; ALVES, C.B.; GONÇALVES, L.M.; DA SILVA, G.W.; OLIVEIRA, B.D.C.; DE ABREU, A.M.; DA CONCEIÇÃO, L.B.; AYUPE, M.C.; DE ARAUJO, M.V.; DE ASSIS, J.B.; DA FONSECA, D.M..
LABORATORY OF MUCOSAL IMMUNOLOGY - INSTITUTE OF BIOMEDICAL SCIENCES OF THE UNIVERSITY OF SÃO PAULO/ INCT MUCOSA AND SKIN, SÃO PAULO - SP - BRASIL.

Mucosal tissues are constantly exposed to external environmental factors, such as pollution, pathogens (viruses and bacteria), and dietary changes, which are frequently used as tools for disease prevention and treatment. However, little is known about the immunological safety of such dietary interventions and the long-term consequences of diet changes for mucosal immunity. In this study, we investigated the immunological impact of the ketogenic diet, a nutritional approach characterized by a high-lipid and low-carbohydrate intake, which shifts the body's energy source from carbohydrates to lipids. Although this diet has therapeutic applications in conditions such as diabetes, obesity, and refractory childhood epilepsy, its indiscriminate use for weight loss has become increasingly common. Data from the Laboratory of Mucosal Immunology showed that mice fed with a ketogenic diet exhibited a marked pulmonary neutrophilic infiltration, which was dependent on IL-6 production and IL-17 receptor expression. We also found an increased infiltration of IL-17-producing gamma delta T cells and innate lymphoid cells (ILCs), which led us to hypothesize that these cells may be a potential mechanism for the recruitment of neutrophils to the lung following the dietary shift. To test this hypothesis we first tested the requirement of T cells (both TCR-alfa/beta and TCR-gamma delta) for the effect of the diet. For that, RAG deficient mice were fed with a ketogenic diet during 5 weeks and the lung inflammatory infiltrate, particularly the neutrophil infiltration, was analyzed by flow cytometry. Based on the results of this experiment, we observed that the phenotype persisted, leading us to hypothesize that the effects of the diet may be related to the IL-17-producing ILC. As the next step, we will investigate how ILCs may contribute to the phenotype observed in response to the ketogenic diet. Keywords: Neutrophilic infiltration; Innate lymphoid cells; Ketogenic diet.

2983 - Category: CELLULAR IMMUNOLOGY (CE)

NEUTROPHIL ACTIVATION UNDER METALLOPROTEINASE ACTION ISOLATED FROM *Bothrops jararacussu* SNAKE VENOM

SILVA, M.D.S.¹; DA SILVA, C.P.¹; DE ARAUJO, V.F.¹; DO ARAÚJO, E.C.S.¹; BRILHANTE, L.M.V.¹; SANTANA, H.M.¹; EULÁLIO, M.D.M.C.¹; SOARES, A.M.¹; SILVA, J.S.²; SETÚBAL, S.D.S.¹; FERREIRA, A.A.F.E.¹; PALOSCHI, M.V.¹; ZULIANI, J.P.³. 1. FIOCRUZ/RO, PORTO VELHO - RO - BRASIL; 2. FIOCRUZ/SP AND USP-FMRP, RIBEIRÃO PRETO - SP - BRASIL; 3. FIOCRUZ/RO AND UNIR, PORTO VELHO - RO - BRASIL.

Snake venom metalloproteinases (SVMPs) from the *Bothrops* genus are proteins with significant local effects in envenoming, but their immunomodulatory effects on neutrophils remain poorly understood. BjussuMP-II, a PI-class SVMP isolated from *Bothrops jararacussu* venom, exhibits proteolytic activity without causing hemorrhage. In this study, the effects of BjussuMP-II on human neutrophil activation were examined, focusing on the NLRP3 inflammasome pathway. Human neutrophils were isolated by density gradient and stimulated with LPS, PMA, or BjussuMP-II (25 µg/mL). Gene expression of inflammasome components (NLRP3, ASC, CASPASE-1, NEK7, IL-1β, GSDMD) and the transcription factors NF-κB and HIF-1α was measured by RT-qPCR. Protein levels were confirmed by Western blot, dot blot, and immunofluorescence. The release of IL-1β, LTB₄, LDH, and extracellular DNA (dsDNA) was also analyzed, alongside cell viability and surface marker expression in murine neutrophils. BjussuMP-II increased gene and protein levels of inflammasome components, promoted ASC/NLRP3 puncta formation, and significantly stimulated the release of IL-1β, LTB₄, LDH, and dsDNA, indicating an inflammatory process with features of pyroptosis. Pre-incubation with the inhibitors MCC950 (NLRP3) and disulfiram (GSDMD) decreased IL-1β release. Neutrophils from *Gsdmd*^{-/-} mice showed reduced IL-1β and dsDNA release, while *HIF-1α*^{-/-} neutrophils showed a selective decrease in IL-1β, suggesting involvement of these components in toxin-induced inflammation. The results demonstrate that BjussuMP-II activates the NLRP3 inflammasome in human and murine neutrophils, contributing to cytokine secretion and NETs formation. This is the first study to show inflammasome activation by a PI-class SVMP, positioning BjussuMP-II as a valuable model for exploring toxin-induced inflammatory mechanisms. Keywords: Metalloproteinase; Neutrophils; Inflammasome.

2985 - Category: CELLULAR IMMUNOLOGY (CE)

Expression of immune checkpoints in regulatory T cells from pregnant women with preeclampsia

DA SILVA, P.B.¹; VEIGA, M.R.²; VASQUES, V.R.R.²; FRANCO, G.D.O.¹; DE OLIVEIRA, L.R.C.²; PERAÇOLI, J.C.¹; PERAÇOLI, M.T.S.³. 1. BOTUCATU MEDICAL SCHOOL, SAO PAULO STATE UNIVERSITY, UNESP, BOTUCATU - SP - BRASIL; 2. INSTITUTE OF BIOSCIENCES OF BOTUCATU, SAO PAULO STATE UNIVERSITY, UNESP, BOTUCATU - SP - BRASIL; 3. INSTITUTE OF BIOSCIENCES OF BOTUCATU, SAO PAULO STATE UNIVERSITY, UNESP, BOTUCATU - SP - BRASIL.

Introduction: Preeclampsia (PE) is a pregnancy-specific syndrome characterized by adaptive immune response shifted toward an inflammatory profile at the expense of the regulatory profile mediated by regulatory T cells (Treg). Molecules called immune checkpoints or anti-inflammatory biomarkers, and tumor necrosis factor receptor 2 (TNFR2) are mainly expressed in Treg cells and play a crucial role in balancing pro- and anti-inflammatory signals that occur at the maternal-fetal interface to ensure maternal tolerance and successful pregnancy. **Objective:** This study aimed to evaluate the immune checkpoints TIGIT, CD155, CTLA-4, PD-1, and GITR, and TNFR2 expression by regulatory T cells in pregnant women with PE. **Materials and methods:** Twenty-four pregnant women were studied, 12 with PE and 12 NT, matched by gestational age. CD4⁺ T cells from peripheral blood were isolated to analyze the expression of TIGIT, CD155, CTLA-4, PD-1, and GITR, and TNFR2 in Treg cells (CD4⁺/CD25⁺/FoxP3⁺) by flow cytometry. The results were analyzed using the Mann-Whitney test with a significance level of 5%. **Results:** Compared to the NT group, pregnant women with PE had a significantly lower percentage of Treg cells ($p < 0.05$). Similarly, the percentage of expression and median fluorescence intensity (MFI) of TIGIT, CTLA-4, PD-1, and TNFR2 in Treg cells was lower in the PE group ($p < 0.05$), although there was no significant difference in TNFR2 MFI. However, the percentage expression of CD155 and GITR was increased in cells of pregnant women with PE, and the MFI was significantly decreased when compared to NT pregnant women ($p < 0.05$). **Discussion and Conclusion:** These findings suggest that preeclampsia is associated with a dysfunctional expression profile of immune checkpoints and TNFR2 in regulatory T cells that may contribute to the loss of maternal-fetal immune tolerance characteristic of the disease. These results indicate an impaired immunoregulatory environment in the pathogenesis of preeclampsia. **Keywords:** Preeclampsia;Regulatory T cells;Immune checkpoints.

3026 - Category: CELLULAR IMMUNOLOGY (CE)

CELLULAR IMMUNE SIGNATURE DISTINGUISHES ACUTE RHEUMATIC FEVER FROM OTHER PEDIATRIC INFLAMMATORY DISEASES

MOREIRA, T.B.¹; ALMEIDA, M.D.²; LUCINDA, P.P.D.²; CAVALCANTE, B.M.²; DE OLIVEIRA, A.J.²; NUNES, M.D.C.P.²; DUTRA, W.². 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Acute rheumatic fever (ARF) is caused by infection with *Streptococcus pyogenes* and mainly affects children aged 3 to 14. Recurrent infections can lead to severe cardiac complications, contributing to an estimated 55 million global cases and around 360,000 deaths annually. Due to its nonspecific inflammatory profile and clinical similarities to other pediatric diseases, ARF is often underrecognized in primary care, leading to underdiagnosis and progression to rheumatic heart disease. Identifying immune biomarkers capable of distinguishing ARF from other inflammatory conditions is thus critical for early diagnosis. In this study, we analyzed fresh whole blood from 16 patients with other inflammatory diseases (CL-CTRL) and 4 with acute ARF, evaluating the frequencies of T cell subsets (CD4⁺, CD8⁺, CD4⁺CD8⁺), B cells, and CD14⁺ monocytes. We also assessed expression of functional markers in these populations. During the acute ARF phase, increased frequencies of CD8⁺, TCRγδ⁺, and CD4⁺CD8⁺PD1⁺ T cells, as well as PDL1⁺ monocytes, were detected. Cell signature analysis showed that CD8⁺, CD4⁺CD8⁺PD1⁺, and CD4⁺CD8⁺TCRγδ⁺ subsets exceeded the diagnostic threshold in over 50% of ARF cases, highlighting their biomarker potential. Monocytes from ARF patients also showed significant alterations in CD86⁺, CD1d⁺, FasL⁺, PDL1⁺, and CD80⁺ expression compared to CL-CTRL. Mean fluorescence intensity analysis revealed higher CD95⁺ levels (a marker of apoptosis) in CD4⁺, CD8⁺, double-negative T cells, and TCRαβ/γδ⁺ populations. These findings suggest a distinct immune profile in acute ARF, with implications for improving diagnostic strategies and preventing progression to chronic heart disease. **Keywords:** Rheumatic fever;Immunophenotyping signature;Biomarkers.

3028 - Category: CELLULAR IMMUNOLOGY (CE)

Circulating T-cell immune profile of patients with rheumatic heart disease with and without previous valvuloplasty

CAVALCANTE, B.M.¹; MOREIRA, T.B.¹; KOH, C.C.¹; NEVES, E.G.A.¹; JACAUNA, A.¹; GOLLOB, K.J.²; CARVALHO, A.T.³; NUNES, M.D.C.P.¹; DUTRA, W.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 3. INSTITUTO RENÉ RACHOU, FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Rheumatic heart disease (RHD) is the most severe sequela of *Streptococcus pyogenes* infection, characterized by chronic, progressive inflammation driven by self-reactive T cells that damage cardiac valves. In this study, we aimed to evaluate circulating T cell subpopulations CD4⁺ and CD8⁺ and their surface marker expression with molecules related to immunoregulation and cellular exhaustion in patients with RHD. Fourteen patients with RHD, with or without a history of percutaneous mitral valvuloplasty, referred for clinical management at the Hospital das Clínicas of UFMG, were recruited for peripheral blood collection. Peripheral blood mononuclear cells (PBMCs) were isolated and stained with surface antibodies for the phenotypic identification of T cell subpopulations and the detection of molecules associated with immunoregulatory and exhaustion profiles of these cell subsets, using multiparametric 28-color flow cytometry using a Symphony BD cytometer. Statistical analyses were performed using GraphPad Prism™; parametric data were assessed via t-tests. No significant differences were observed in total CD4⁺ or CD8⁺ T cell frequencies or in exhaustion markers. However, within the CD8⁺ subset, the regulatory marker CD185⁺ was significantly more expressed in patients who had not undergone valvuloplasty. Furthermore, in the double-negative T cell subset (CD4⁻CD8⁻), patients without valvuloplasty exhibited higher expression of regulatory markers CD152, CD196, and CD294, as well as the exhaustion marker CD366. These findings show distinct immunological profiles in RHD patients depending on surgical intervention history, indicating that patients without intervention have a less intense inflammatory profile when compared to patients who have undergone intervention. These data could inform targeting strategies for disease monitoring, prognosis, and targeted immunomodulatory therapies. Keywords: Rheumatic heart disease; T cells; immunoregulation.

3041 - Category: CELLULAR IMMUNOLOGY (CE)

Role of Neutrophil Extracellular Vesicles in the Control of Chikungunya Virus Infection

GONÇALVES, M.; DA SILVA, J.O.; MATTOS, T.R.F.; WACLAWIAK, I.; COELHO, S.V.A.; COSTA, L.; COSTA, A.G.B.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Arboviruses can cause diseases ranging from mild fever to severe, potentially fatal outcomes. According to the WHO, arboviruses such as Chikungunya (CHIKV) pose ongoing public health threats in tropical and subtropical regions. Although neutrophil recruitment occurs early during infection, experimental models still poorly address the role of these cells and their extracellular vesicles (nEVs) in CHIKV infection. EVs are lipid bilayer-enclosed, non-replicative particles released by cells, capable of transporting proteins, nucleic acids, lipids, and metabolites. nEVs are emerging as key modulators of inflammation and immune responses. This study investigates the role of nEVs in CHIKV infection of human macrophages. Neutrophils and PBMCs were isolated from donor blood by density gradient. Neutrophils were incubated in serum-free RPMI for 1h at 37 °C. Small EVs (SEVs) and large EVs (LEVs)-enriched fractions were obtained by ultracentrifugation. NTA and electron microscopy showed LEVs ranged from 250–360 nm and SEVs around 150 nm. Protein content was measured by BCA assay. Monocytes were differentiated into macrophages with 10% human serum for seven days. CHIKV infection was tested using MOIs of 0.01, 0.1, and 1; MOI 0.1 was chosen for further assays. To assess the impact of nEVs, macrophages were infected with CHIKV (MOI 0.1) in the presence or absence of LEVs or SEVs for 1h30. After 24h, supernatants were collected for plaque assays in VERO cells. Our data show that LEVs completely block CHIKV infection in human macrophages. Ongoing experiments will explore the underlying molecular mechanisms involved in the observed interference. We plan to evaluate the expression of genes related to the antiviral and inflammatory response, the quantification of pro- and anti-inflammatory cytokines in culture supernatants, cell viability, and the production of reactive oxygen species (ROS) in infected macrophages in the presence or absence of nEVs. Keywords: Extracellular vesicle; Chikungunya infection; arboviruses.

3048 - Category: CELLULAR IMMUNOLOGY (CE)

Development of Cellular Immunotherapies for Gastric Carcinomas

STEIMBACH, J.V.¹; KHAYAT, A.S.²; DE ASSUMPÇÃO, P.P.²; BONAMINO, M.H.¹. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO PARÁ, BELÉM - PA - BRASIL.

Gastric cancer is the fifth most common and deadly malignancy worldwide. Curative treatment relies on gastrectomy combined with chemotherapy and monoclonal antibodies. Since the 2000s, cellular therapies such as in vitro-expanded tumor-infiltrating lymphocytes (TIL) and anti-Claudin 18.2 CAR-T cells have been tested in advanced cases, but with limited success. Like most solid tumors, CAR-T cells in gastric cancer show low persistence and expansion. This project aims to identify therapeutic targets in Brazilian patients and develop cell therapy products from healthy donors' lymphocytes or patient-derived TIL to elicit strong, durable antitumor responses. Patient transcriptomics will be evaluated to explore targetable immune interactions. CRISPR/Cas9 gene-editing will be used to disrupt PDCD1, HAVCR2, CTLA4, and LAG3 expression in anti-Claudin 18.2 CAR-T cells and TIL. Preliminary RNA-seq analysis of 120 Brazilian patients revealed differential expression of tumor microenvironment-related genes. In primary tumors, CTLA4 and HAVCR2 were significantly upregulated compared to adjacent tissues. In peritoneal metastases, expression of CD274, LGAL9, FGL1 (encoding ligands for PD-1, TIM-3, and LAG-3 respectively), and HAVCR2 was increased. Additionally, CLDN18.2 was predominant over isoform 1, highlighting its potential as an immunotherapy target. Notably, anti-Claudin 18.2 CAR-T cells were already successfully generated using CRISPR/Cas9 to knock in the CAR construct at the PDCD1 or CTLA4 loci, ensuring endogenous gene disruption in CAR+ cells. Additionally, as a proof of concept, TIL were successfully isolated and expanded from the ascitic fluid of a gastric cancer patient, demonstrating the feasibility of TIL-based strategies in this context. This project aims to enhance cell resistance and expansion in the suppressive tumor microenvironment, advancing cellular therapy for gastric cancer, with the goal of improving patient outcomes. Keywords: Cell Therapy; Gastric Cancer; Inhibitory Receptors.

3062 - Category: CELLULAR IMMUNOLOGY (CE)

Metabolic syndrome is associated with imbalance of circulating B-cell subsets

GONÇALVES, H.C.; OYAMADA, H.A.D.A.; BILHÃO, R.; DE MORAES, D.B.C.; PERCEGONI, A.C.R.; PORTELA, J.V.R.; DO SACRAMENTO, P.M.; BENTO, C.A.D.M.. UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Characterized by obesity and alterations in several metabolic parameters, such as blood pressure, total triglycerides and total HDL, people with metabolic syndrome (pwMetS) have been shown to be more susceptible to hypersensitivity reactions and severe infectious diseases. Moreover, they also present with abnormal responses to vaccination against different pathogens, showing an inferior immune response after the prophylaxis. This adverse event is associated with defects in B cells, however, there is few studies evaluating the effect metabolic syndrome on this lymphocyte population in humans. As such, the present study aims to evaluate the phenotypic profile of B cells in obese individuals with or without MetS. According to the differential expression of CD3, CD19, CD20, IgD, CD38, CD27, HLA-DR, as well as IL-10, IL-17 and IFN- γ , the frequency of different circulating B-cell (CD19⁺) subsets were evaluated using flow cytometry. In the present study, pwMetS showed lower frequency of different memory B cells with HLA-DR⁺IgD⁺ and HLA-DR⁺IgD⁻ phenotypes, although the intensity of HLA-DR expression in these cells was higher in sample from pwMetS. Interestingly, memory HLA-DR⁺IgD⁺ B cells, a phenotype classically associated with anergic B cells, were higher in pwMetS. However, in contrast to the anergic B cells, both IL-10 production and intensity of IgD expression in HLA-DR⁺IgD⁺ B cells were lower in pwMetS. Although preliminary, our findings suggest that increased circulating abnormal anergic B cells associated with immunogenic B cell subsets imbalance in pwMetS could not only contribute to higher risk in infection diseases, but also favor development or exacerbation of autoimmune diseases. Keywords: B cell; Metabolic Syndrome; Autoimmune diseases.

3063 - Category: CELLULAR IMMUNOLOGY (CE)

Phenotyping of dendritic cells differentiated from murine bone marrow in the presence of GM-CSFDE SIQUEIRA, P.F.; VALERIANO, J.D.P.; SILVA, M.F.D.S.C.. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL.

Dendritic cells (DCs) are key components of innate immunity and main activators of adaptive immune responses due to their ability to migrate to secondary lymphoid organs and prime T lymphocytes. DCs excel in antigen capture, processing, and presentation through major histocompatibility complexes (MHC) I and II, which, along with co-stimulatory signaling and the production of multiple cytokines, are capable of inducing varied lymphocyte activities. In 1999, Lutz et al. described a method to differentiate murine bone marrow cells into DCs using GM-CSF, which became one of the most used methods for studying DC biology. However, this method is questioned on its efficiency in representing cells *in vivo* for their phenotype and heterogeneity of the population. Therefore, we analyzed the phenotypical and transcriptional profiles of these cells through flow cytometry and public single-cell RNA sequencing (scRNA-seq) data. Cells were cultivated with different GM-CSF concentrations (5, 10, and 20 ng/ml) to assess ideal conditions and were evaluated on different days (7, 10, and 14 days). We observed widespread expression of CD11c and MHC II, especially after the 10th day. We also determined that the ideal settings for obtaining DCs are 10 ng/ml for 10 days, which yields more cells and a higher percentage of immature DCs. scRNA-seq analysis showed that these cells are indeed DCs; but there are also macrophages being generated, although mostly adhered to the plate. We also investigated the DC subtypes generated and observed some were monocyte-derived DCs while others were similar to conventional DCs; however, these cells could not be associated with their *in vivo* counterparts, which corroborates the literature by stating they do not fully represent *in vivo* DCs. This study contributes to our understanding of DCs by highlighting the need for a more thoughtful interpretation of results and by investigating better culture conditions to improve the data obtained using this model. Keywords: Dendritic cell; Bone marrow; Cell differentiation.

3065 - Category: CELLULAR IMMUNOLOGY (CE)

Spectral Immunophenotyping for Diagnostic Support in Inborn Errors of ImmunityMATIAS, D.O.¹; GANDINI, M.¹; DE MELO, M.A.F.¹; DOS SANTOS, C.R.¹; FIGUEIREDO, V.¹; COLLI, B.²; PONTILLO-GUIMARÃES, G.²; RIZZO, L.³; DE VASCONCELOS, Z.F.M.⁴; PEREIRA, G.M.B.¹. 1. FIOCRUZ-RJ, RIO DE JANEIRO - RJ - BRASIL; 2. THERMOFISHER SCIENTIFIC, SÃO PAULO - SP - BRASIL; 3. THERMOFISHER SCIENTIFIC, SÃO PAULO - SP - BRASIL; 4. FIOCRUZ RJ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Inborn errors of immunity (IEI) comprise a heterogeneous group of genetic disorders that affect immune system development and function. Early screening and phenotypic characterization of leukocytes are essential for clinical decision-making and guiding molecular investigation. **Aim:** We standardized a high-parameter spectral flow cytometry panel using whole blood for extended immunophenotyping to support IEI diagnosis. **Materials and methods:** A whole blood sample from a healthy adult female donor (38 years old) was used for the optimization of spectral flow cytometry. Blood (4 mL in EDTA) was processed using ACK lysing buffer (Thermo Fisher Scientific), with 100 µL used per staining tube. The 27-marker panel included T cell markers (CD3, CD4, CD8, CD45RA, CD27, CD28, CD25, CD127), B cells (CD19, CD27, IgD, CD38), NK cells (CD56, CD16), and myeloid lineage markers (CD14, CD16, CD11c, CD123, HLA-DR, CD66b, BDCA-2, Siglec-8). Samples were acquired on the Bigfoot Spectral Cell Sorter and analyzed using FlowJo v10.10.0. **Results:** The panel enabled detailed identification of 25 immune subpopulations, including T cell maturation subsets (naïve, memory, terminal effector memory re-expressing RA), B cell developmental stages, NK cell subsets, neutrophils, eosinophils, monocytes (classical, intermediate, non-classical), and dendritic cells. **Conclusion:** This configuration allowed high-resolution immune profiling using minimal blood volume, proving robust, reproducible, and suitable data for pediatric clinical application and early IEI screening. Keywords: : Inborn Errors of Immunity; Spectral Flow Cytometry; Whole Blood Immunophenotyping.

3083 - Category: CELLULAR IMMUNOLOGY (CE)

Immunological Signatures Associated with Bone Loss in People Living with HIV/AIDS (PLWHA)

PRAXEDES, I.R.¹; NOGUEIRA, P.A.¹; PINHEIRO, R.D.S.¹; XAVIER, T.D.S.¹; DA SILVA, A.Q.P.²; FERREIRA, S.C.³; CHAVES, Y.O.¹. 1. INSTITUTO LEÔNIDAS E MARIA DEANE/FIOCRUZ AMAZÔNIA (ILMD/FIOCRUZ AMAZÔNIA), MANAUS - AM - BRASIL; 2. UNIVERSIDADE FEDERAL DO AMAZONAS (PPGIBA/UFAM), MANAUS - AM - BRASIL; 3. CENTRO UNIVERSITÁRIO FAMETRO, MANAUS - AM - BRASIL.

The increased life expectancy of people living with HIV/AIDS (PLWHA), driven by antiretroviral therapy (ART), has been accompanied by age-related complications such as decreased bone mineral density (BMD). As the immune system plays a central role in bone remodeling, particularly via CD4⁺ T cell subpopulations, HIV-associated chronic inflammation may lead to early bone loss and higher fracture risk. In this cross-sectional study, we evaluated BMD, clinical parameters, and CD4⁺ T cell immunological profiles in 50 PLWHA over 40 years of age. After informed consent, participants underwent dual-energy X-ray absorptiometry (DXA) and peripheral blood collection for flow cytometry. Of the 48 individuals analyzed, 12 had normal BMD, 23 had osteopenia, and 13 had osteoporosis, with a male predominance. Men with osteopenia showed increased frequencies of CD4⁺Ki-67⁺ T cells, while PLWHA with bone loss presented reduced frequencies of CD4⁺CTLA-4⁺ T cells. Additionally, activation markers HLA-DR and CD69 were upregulated in CD4⁺CTLA-4⁺ subsets in these groups. In cytokine analysis, osteopenic men exhibited lower IFN- γ and higher TNF- α production. Our findings suggest that immune activation and regulatory T cell imbalance contribute to bone loss in PLWHA and highlight the importance of sex-specific immune markers as potential targets for intervention. Keywords: HIV; Bone loss; CD4⁺ T cells.

3088 - Category: CELLULAR IMMUNOLOGY (CE)

Uncovering the Role of p53 in Regulatory T Cell Biology

BARBOSA, G.A.D.C.; DAMASCENO, L.E.A.; DE MELO, B.M.S.; LEANDRO, M.D.O.; SEVERINO, M.B.; BRUNINI, G.L.; SILVA, B.M.S.; CUNHA, F.Q.; CUNHA, T.M.; ALVES-FILHO, J.C.. RIBEIRAO PRETO MEDICAL SCHOOL - UNIVERSITY OF SAO PAULO, RIBEIRAO PRETO - SP - BRASIL.

The p53 protein plays key roles in the cellular stress response by regulating cell fate through mechanisms such as cell cycle arrest, senescence, DNA repair, and apoptosis. Regulatory T cells (Treg), a CD4⁺ T cell subset with immunosuppressive functions, are essential for immune regulation and tolerance. While evidence suggests p53 contributes to immune homeostasis, its specific role in Treg biology and function remain poorly understood. In this study, we propose to evaluate the role of p53 in Treg cell differentiation and function and its impact on immune homeostasis. Our results demonstrate that the pharmacological activation of p53 (Nutlin-3) enhanced iTreg cell differentiation in vitro, as evidenced by the higher Foxp3 expression levels. In line, iTreg cell differentiation was impaired in the absence of p53 (CD4^{Cre}p53^{Flox/Flox}), implying a role of p53 in driving Treg cell development. The process of aging accompanies alterations in Treg cell functions that contribute to the disruption of immune system homeostasis. Interestingly, Treg-specific p53-deficient aged mice (10 months) exhibited a decreased proportion of naïve T cells, while the population of activated T cells was increased, alongside elevated IFN- γ production by CD4⁺ and CD8⁺ T cells, suggesting functional deregulation. We then tested whether p53 deficiency in Treg cells could account for alterations in their function within a tumor context. Using the MC38 ectopic model, we observed that Foxp3^{Cre}p53^{Flox/Flox} mice showed reduced tumor growth compared to controls, indicating that the deleterious role of Treg in this context was overridden by p53 deletion, thereby enhancing antitumor responses. Taken together, our findings reveal that p53 is a key regulator of Treg cell differentiation and function in both homeostasis and tumor contexts, highlighting it as a target for Treg cell-directed therapeutic approaches. Keywords: p53; Treg; Immune response.

3089 - Category: CELLULAR IMMUNOLOGY (CE)

Characterization of CD18 Localization and Function in Virus-Containing Compartments of Mature Dendritic Cells and U937-CD169⁺ Cells

CAMARGO, F.E.C.; SOUZA, K.B.D.S.; DE ALENCAR, B.C.. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Dendritic cells (DCs) express HIV-1 recognition receptors but are rarely productively infected. Mature DCs can sequester intact virions into virus-containing compartments (VCCs) via SIGLEC1 (CD169), which binds viral envelope gangliosides. These cells mediate trans-infection, enhancing viral spread. Although SIGLEC1's role in viral capture is known, its short cytoplasmic tail lacks internalization motifs, and its role in VCC formation remains unclear. CD18 (β_2 integrin), which dimerizes with CD11 and is present in macrophage VCCs, may contribute to compartment stabilization or signaling. Therefore, the objective of this work was: develop a model of VLP-GFP⁺ VCC formation in THP-1 or U937 cells expressing SIGLEC1; confirm CD18 presence in VLP-GFP⁺ VCCs of mMoDCs and CD169⁺ cell lines; and analyze VCC formation in mMoDCs silenced for CD18 and in U937-CD169⁺ CD18-knockout cell line. Lentiviral vectors were produced in HEK293T cells treated with 1.3 mM 3'-sialyllactose and transfected with pLVCD169, psPax2, and pVSV-G. Transduction in THP-1 cells was unsuccessful, shifting focus to U937 cells, which reached 78% efficiency. Transduced cells were sorted for CD169 expression and expanded under antibiotic selection. A VLP-GFP⁺ internalization assay was performed in both cell lines for immunofluorescence assay. VCCs were quantified and visualized using wildfield and confocal microscopy. Five shRNAs targeting CD18 were tested in mMoDCs, but just three showed performance and was low. A stable U937-CD169⁺ model capable of forming VCC-like structures was established, mimicking mMoDCs. CD18 was detected in VCCs of both mMoDCs and U937-CD169⁺ cells, though its distribution remains undefined. CD18 silencing effects are still unclear, but the enhanced transduction or CRISPRi are under evaluation. Next, CD18 conformational states and roles in VCC formation will be assessed. Understanding these mechanisms may reveal new therapeutic targets for HIV-1 and other CD169-binding pathogens. Keywords: HIV-1;Dendritic-cells;Siglec-1.

3099 - Category: CELLULAR IMMUNOLOGY (CE)

Lung-Gut Axis: Role of the Axl Receptor in COPD-Associated Intestinal Inflammation

RIBEIRO, J.D.O.; PINTO, K.G.; DA COSTA, A.L.A.; FERREIRA, J.R.M.; NASCIMENTO, J.G.; MENDES, C.M.; DE LIMA, J.V.C.S.; DE SÁ, M.C.M.D.M.A.; MARINS, J.D.G.; SANTOS, J.M.; PIMENTA, A.J.; FILARDY, A.D.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Chronic Obstructive Pulmonary Disease (COPD) is a progressive respiratory disease mainly associated with cigarette smoke. Besides the lungs, COPD affects other tissues, such as the intestines, contributing to inflammation, increased permeability, and dysbiosis. Cigarette smoke-induced inflammation causes cell death, making efferocytosis essential to restore tissue balance. The TAM receptors — Tyro3, Axl, and MerTK — mediate the recognition of apoptotic cells, regulating pro-inflammatory pathways. Although the role of these receptors in lung diseases has been explored, their impact on the lung-gut axis during COPD has not been addressed. Therefore, we investigated Axl receptor-mediated efferocytosis in the susceptibility of individuals with COPD to intestinal inflammation. Wild-Type (WT) and Axl-deficient (Axl^{-/-}) C57/BL6 mice from the COPD group were exposed to cigarette smoke three times daily, five days a week for three months. Mice from the RA (control) group were exposed to room air. Colons from WT and Axl^{-/-} mice were collected to analyze immune cell frequency in the lamina propria by flow cytometry, and colon homogenates were used for cytokine determination by ELISA. Increased numbers of colonic macrophages, monocytes, and neutrophils were observed in the lamina propria of Axl^{-/-}-COPD mice compared with their control group, and even higher than the WT-COPD group. Furthermore, colon homogenates from Axl^{-/-}-COPD mice had higher TNF levels than Axl^{-/-}-RA mice. No changes in IL-17 levels were observed. Finally, Axl^{-/-}-COPD mice showed reduced IL-1 β levels compared to WT-COPD mice. These findings suggest that COPD may represent a risk factor for higher susceptibility to inflammatory bowel conditions and indicate that the Axl receptor may play a relevant role in modulating intestinal inflammation. Keywords: Chronic Obstructive Pulmonary Disease (COPD);efferocytosis;lung-gut axis.

3113 - Category: CELLULAR IMMUNOLOGY (CE)

Evaluation of CAR-T Cell Persistence and Migration Using the Antares System in a Murine Model

NEUMANN, A.D.S.; ABDO, L.D.M.; ARAGAO, E.A.A.; BONAMINO, M.H.. INSTITUTO NACIONAL DO CÂNCER - INCA, RIO DE JANEIRO - RJ - BRASIL.

CAR-T cells represent a promising immunotherapy strategy against various types of cancer. Bioluminescence assays in animal models using tumor cell lines with luciferase expression are widely employed to evaluate the effectiveness of CAR-T cell treatments. However, the persistence and migration patterns of these CAR-T cells in vivo is poorly known. The Antares system (AS), an improved version of NanoLuc, stands out for its small size and high sensitivity, enabling non-invasive monitoring of cells in in vivo models. Therefore, this project aims to evaluate the migration and persistence of CAR-T cells containing AS (CAS), produced via PiggyBac plasmid vectors, in immunodeficient mice inoculated with the Nalm-6 tumor cell line expressing the Firefly system (FS). When the tumor reached a detectable level by FS (13 days post-inoculation), animals received 10^6 CAS cells, and both the recommended and half doses of substrate generated strong and consistent bioluminescent signals. In another group, treated after only 2 days of tumor growth (undetectable by FS), a lower CAS bioluminescent signal was observed, possibly due to lower tumor burden and, consequently, reduced CAR-T cell expansion. Additionally, it was confirmed that the AS substrate does not interact with FS, used by Nalm-6 cells. In another experiment, animals treated after 2 and 9 days of tumor progression showed that CAR-T cell proliferation and migration are proportional to tumor progression time, suggesting greater CAS expansion in response to higher tumor burden. Therefore, our study demonstrates the feasibility of simultaneously tracking CAR-T and Nalm-6 cells using different systems in the same experiment, enabling a better understanding of CAR-T cell distribution in various contexts. Keywords: CAR-T;Antares;Luciferase.

3116 - Category: CELLULAR IMMUNOLOGY (CE)

Platelet–Leukocyte Interactions Shape Inflammation and Antibody Responses in a Murine Model of Allergic Asthma

DE SOUZA, V.P.¹; MENEGATI, L.M.²; DE OLIVEIRA, E.E.²; DIB, P.R.B.³; MACHADO, L.F.¹; ALMEIDA, A.P.G.²; ALVES, D.C.D.A.²; DURAO, C.V.²; IZOLAN, L.B.²; SILVA, R.S.F.²; DA SILVA, A.B.R.²; MACEDO, G.C.⁴; HOTTZ, E.D.¹; SILVA, F.M.D.C.E.². 1. UFJF, JUIZ DE FORA - MG - BRASIL; 2. UERJ, RIO DE JANEIRO - RJ - BRASIL; 3. UFJF, JUIZ DE FORA - - MG - BRASIL; 4. UFJF, RIO DE JANEIRO - MG - BRASIL.

Allergic asthma is a chronic inflammatory disease of the airways characterized by a Th2-skewed immune response, marked eosinophilia, and elevated IgE production. Recent evidence indicates that, beyond their classical role in hemostasis, platelets also participate in inflammatory and immune processes by interacting with leukocytes and modulating adaptive immunity. However, their role in allergic asthma remains incompletely understood. This study aimed to investigate the role of platelets in the development of the allergic response in secondary lymphoid organs and in pulmonary inflammation using a murine model. Female Balb/c mice were sensitized and challenged with ovalbumin (OVA) and treated with anti-CD41 to induce platelet depletion. During the allergic immune response in the lungs, increased levels of IL-5, CCL11, and PF4 were observed, along with local and systemic eosinophilia. Under these conditions, enhanced formation of platelet–leukocyte aggregates was detected, particularly with eosinophils, alveolar macrophages, and B cells. Partial platelet depletion with anti-CD41 treatment led to a reduction in plasma PF4, eosinophilic infiltration, and the formation of platelet–leukocyte aggregates in the lungs. In parallel, Th2 lymphocytes were reduced in the mediastinal lymph nodes. The treatment also affected B cell subpopulations, with a decrease in IgG1⁺ B cell–platelet aggregates and an increase in IgE⁺ B cells in peripheral lymphoid organs, depending on the protocol phase. Although serum levels of these antibodies were not altered, their functional quality was impaired, as evidenced by reduced avidity and impaired lymphoid follicle formation in the spleen. These findings demonstrate that platelets actively participate in allergic asthma by modulating various aspects of the immune response, with context-dependent effects depending on the phase of inflammation. Keywords: Asthma;platelets;immune response.

3117 - Category: CELLULAR IMMUNOLOGY (CE)

TISSUE-RESIDENT MEMORY T CELLS IN TRACHEAL ASPIRATES AS BIOMARKERS OF PULMONARY DISEASES

DE ANDRADE, G.P.; GONÇALVES, J.V.P.; DE MOURA, B.I.K.; DRUMM, M.E.G.O.; MILESI, L.M.; SEADI, R.D.; HAUBERT, A.F.; RODRIGUES JUNIOR, L.C.. UFCSPA, PORTO ALEGRE - RS - BRASIL.

Tissue-resident memory T cells (TRMs) are a subset of lymphocytes that remain permanently localized in non-lymphoid tissues, where they play a pivotal role in local immunosurveillance. In the lungs, TRMs contribute to immune defense against pathogens, tumors, and other diseases. CD4⁺ and CD8⁺ TRMs have emerged as potential biomarkers for disease involvement and prognosis in various pulmonary conditions, but their characterization typically requires invasive procedures such as biopsies. Notably, TRMs are also present in the tracheal epithelium, raising the potential for less invasive sampling. **Objectives:** This study aimed to characterize TRMs and other memory T cell subsets in tracheal aspirates from intubated patients to evaluate this fluid as a non-invasive source for monitoring pulmonary immune status.

Methods: Aspirates from patients with or without lung disease were processed using five protocols: (1) Ficoll density gradient, (2) 0.5% N-acetylcysteine (NAC), (3) 0.1% dithiothreitol (DTT), (4) combined NAC and DTT, and (5) PBS/EDTA wash. Cells were stained with a viability dye and antibodies against CCR7, CD3, CD4, CD8, CD45RA, CD103, and CD69, followed by flow cytometry. Confocal microscopy was also used to detect lymphocytes labeled with CD3 and CD103. **Results:** From the 26 patients, the DTT-based protocol yielded the best results, with an average of 1.28×10^9 cells and 93.15% viability. Lymphocytes averaged 25.7% of the population, with CD4 TRMs representing 17.1% and CD8 TRMs 51.35%. Patients with infectious pneumopathies showed increased CD4 and CD8 effector memory T cells relative to TRMs. Confocal microscopy confirmed TRM presence. **Conclusion:** These findings support the use of tracheal aspirates to differentiate memory T cell subsets in pulmonary diseases. **Keywords:** tissue-resident memory T cells;tracheal aspirates;confocal microscopy.

3121 - Category: CELLULAR IMMUNOLOGY (CE)

Longitudinal Systemic Immune Profiles Associated with Clinical Response in Metastatic NSCLC PatientsFERREIRA, T.R.¹; BARBETA, M.G.D.O.¹; FERREIRA, S.M.I.¹; APOSTOLICO, J.D.S.¹; EVANGELISTA, G.F.D.B.¹; FREITAS, H.C.²; DE LIMA, V.C.C.²; GROSS, J.L.²; FIGUEIREDO, A.B.¹; GOLLOB, K.J.¹. 1. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. AC CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

Clinical responses to immunotherapy in metastatic non-small cell lung cancer (NSCLC) vary widely and remain poorly understood. This study aimed to uncover immune mechanisms associated with therapeutic outcomes by characterizing longitudinal changes in plasma immune mediators and peripheral immune cells. Fresh blood samples were collected at three time points: before treatment (T0), three weeks after starting immunotherapy (T1), and nine weeks after treatment began (T2). Plasma cytokines, chemokines and growth factors were measured using multiplex bead-based assays, and immune cell populations were analyzed by multiparametric flow cytometry. Among responders (R), we observed a progressively regulated inflammatory profile marked by a sustained decrease in IL-4/IL-13 and a gradual increase in IL-18, as well as changes in chemokines (e.g., recovery of MCP-1 and rising MIG). Responders also showed expansion of immune-activating subsets, including increased frequencies of CD4⁺ T cells and plasmablasts, and higher HLA-DR expression (MFI) on B cells and monocytes. In contrast, non-responders (NR) maintained a dysregulated immune state, with persistently elevated proinflammatory cytokines (e.g., IL-6, IFN- α 2), chemokines (e.g., IL-8, MIP-1 β , MCP-3), and growth factors (e.g., FGF2, M-CSF). Despite early immune activation, these responses were not sustained (e.g., decreasing CTLA4⁺ T cells), and were accompanied by reduced plasma IL-10 and IL-7. These longitudinal data reveal systemic immune dynamics that distinguish responders from non-responders. **Funding:** Supported by FAPESP/GSK (grant #21/00408-6). **Ethical approval:** Approved by the Research Ethics Committee of Hospital Israelita Albert Einstein (CAAE: 54281321.6.0000.0071). **Keywords:** Immunotherapy;Cytokines;Lung Cancer.

3134 - Category: CELLULAR IMMUNOLOGY (CE)

Systemic immune profiling by Single-cell analysis associated with treatment response in metastatic NSCLC

BARBETA, M.G.D.O.¹; FERREIRA, T.R.¹; FIGUEIREDO, A.B.¹; FERREIRA, S.M.I.¹; MORAIS, K.L.P.¹; EVANGELISTA, G.F.D.B.¹; FREITAS, H.C.²; DE LIMA, V.C.C.²; GROSS, J.L.²; GOLLOB, K.J.¹. 1. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. AC CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

Background: The systemic immune landscape linked to therapeutic response in metastatic non-small cell lung cancer (NSCLC) remains poorly understood. Uncovering the molecular and cellular mechanisms associated with clinical outcomes may help identify predictive biomarkers and guide the development of new treatment strategies.

Methods: We performed single-cell transcriptomic analysis of peripheral blood mononuclear cells (PBMCs) isolated from stage IV NSCLC patients prior to treatment. Patient response was determined at 6 months following therapy. Individual cells were annotated and analyzed using bioinformatics tools in Python to identify differentially expressed genes between responders and non-responders.

Results: In non-responders, PBMCs showed upregulation of genes linked to cell death and innate immune activation, including *NGFR* and *HLA-DR* genes. In contrast, responders exhibited increased expression of genes associated with tumor suppression, such as *IGFBP4*. Further analysis indicated that responders may have a stronger systemic Th1-mediated immune response, while non-responders displayed a more generalized innate immune activation. Therapeutic response also appeared to correlate with a more regulated immune profile, potentially avoiding excessive immune activation.

Conclusions: These results suggest that responders to therapy exhibit a more targeted and regulated systemic antitumor immune response, whereas non-responders are characterized by a less specific, innate immune activation profile.

Funding: Supported by FAPESP/GSK (grant number #21/00408-6).

Ethical approval: This study was approved by the Research Ethics Committee of the Hospital Israelita Albert Einstein (CAAE: 54281321.6.0000.0071; Approval Number: 6.821.782).

Keywords: Lung cancer;Single-cell sequencing;Immunotherapy.

3138 - Category: CELLULAR IMMUNOLOGY (CE)

Study of the regulation of gut microbiota composition by AXL and MERTK receptors in animals exposed to cigarette smoke

NASCIMENTO, J.G.¹; DA COSTA, A.L.A.¹; RIBEIRO, J.D.O.¹; DE LIMA, J.V.C.S.²; DE SÁ, M.C.M.D.M.A.¹; MENDES, C.M.¹; MARINS, J.D.G.¹; SANTOS, J.M.³; PIMENTA, A.J.⁴; PINTO, K.G.¹; FERREIRA, J.R.M.¹; FILARDY, A.D.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIRIO, RIO DE JANEIRO - RJ - BRASIL; 3. UFRRJ, RIO DE JANEIRO - RJ - BRASIL; 4. UERJ, RIO DE JANEIRO - RJ - BRASIL.

Smoking is a major risk factor for acute and chronic diseases, including cancer and immune-mediated inflammatory disorders (1). Beyond the lungs, cigarette smoke (CS) affects extra-pulmonary tissues and contributes to intestinal disorders by inducing inflammation, increased permeability, impaired digestion and promoting dysbiosis (2). The crosstalk between pulmonary and intestinal mucosa may explain intestinal diseases in smokers and patients with chronic lung conditions; however, the mechanisms remain unclear (3). Understanding how immune responses are regulated during smoking could help manage intestinal disorders in smokers. CS-induced inflammation triggers cell death, requiring clearance of apoptotic cells by phagocytes through efferocytosis to prevent necrosis and restore homeostasis. Macrophages express receptors that recognize apoptotic cells, including the TAM family (Tyro3, Axl, and MerTK), which bind phosphatidylserine via bridging molecules. TAM activation regulates efferocytosis and inflammation by inducing expression of regulatory proteins. Here, we investigated the role of Axl and MerTK in gut microbiota modulation in animals exposed to CS. Wild-type (WT) C57BL/6 mice and Axl or MerTK knockout mice were exposed to smoke from nine cigarettes in three daily sessions for five days. Fecal samples underwent DNA extraction and 16S rRNA gene (V3–V4) sequencing via Illumina MiSeq. CS exposure altered gut microbiota in knockouts, with reduced Bacteroidota and increased Desulfobacterota at the phylum level. At the genus level, *Hungatella* and *Klebsiella* increased in knockouts, while *Phocaeicola* predominated in WT mice. No major differences appeared in WT animals exposed to CS alone. These preliminary findings suggest Axl and MerTK are critical for maintaining gut microbial balance, and their absence may heighten susceptibility to smoking-related infections. **Keywords:** Efferocytosis;Lung–gut axis;Dysbiosis.

3150 - Category: CELLULAR IMMUNOLOGY (CE)

CBX4 MODULATION IN CD8 T CELLS DURING ACUTE INFECTION AND CANCER

NETO, M.A.N.¹; MELO, G.A.¹; PASSOS, T.D.O.¹; CHAGAS-NETO, A.J.¹; OLIVEIRA, J.P.L.¹; VIEIRA, B.L.M.D.O.²; HIGA, L.¹; BERBERT, L.¹; VIOLA, J.P.D.B.²; WERNECK, M.B.D.F.¹; NETO, H.A.D.P.¹; LIMA, J.E.N.¹; PEREIRA, R.D.M.S.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL.

Cytotoxic CD8 T cells (CTLs) play a central role in eliminating intracellular pathogens and tumor cells. During acute infections, CTLs differentiate into a heterogeneous population consisting of short-lived effector cells and various memory subsets (Nat. Rev. Immunol. (2012) 12:749). We previously demonstrated a novel role for the epigenetic modulator Chromobox (Cbx) 4 in regulating CTL differentiation. Cbx4 can be part of the Polycomb Repressive Complex 1 (PRC1), which represses gene expression by ubiquitinating lysine 119 on histone H2A (H2AK119ub) through the catalytic subunit Ring1A/B. Cbx4 recognizes trimethylated lysine 27 on histone H3 (H3K27me3), helping recruit PRC1 to these sites (Cell (2007) 128:735). We infected C57BL/6 Cbx4^{fl/fl} CD4Cre (Cbx4 T KO) mice, which are deficient in Cbx4 in the T-cell compartment, with the acute LCMV Armstrong 5 strain (CEUA/UFRJ 003/23). This resulted in an increased frequency of virus-specific memory precursor cells, suggesting that Cbx4 regulates CTL stemness in acute infection. However, effector function was preserved in CTLs from Cbx4 TKO mice, as serum LCMV titers were undetectable on day 7 post-infection. Furthermore Cbx4 TKO mice have improved tumor protection in a B16 melanoma model (CEUA/UFRJ 041/20), with an increased proportion of central memory T cells. In addition, Cbx4-silenced CTLs (shCbx4) cultured with IL-15 and low-dose IL-2 were transferred into B16 melanoma-bearing mice. Tumor-infiltrating lymphocytes (TILs) from animals that received adoptive transfer of shCbx4 CTLs showed increased expression of memory markers, which is desirable in immunotherapy strategies. Therefore, our findings suggest that while Cbx4 deletion commits CTLs to a memory precursor phenotype, it does not compromise acute viral clearance. Moreover, Cbx4 deletion promotes a progenitor-like T cell state with the potential to enhance long-term tumor control in immunotherapy settings

Keywords: CD8 T Cells;Cbx4;Immunotherapy.

3153 - Category: CELLULAR IMMUNOLOGY (CE)

Advancing CAR-T Cell Engineering: Hybrid Transposons as Cost-Effective non-Viral Alternatives

ABDO, L.D.M.; ARAGAO, E.A.A.; NEUMANN, A.D.S.; CORREIA, E.M.; SILVA, L.R.B.; BONAMINO, M.H.. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

CAR-T cell therapy is a major advance in treating B cell tumors, but its high cost—partly due to viral vectors—limits access, highlighting the need for alternative gene modifying tools. In this study, we used transposon-based tools called Sleeping Beauty (SB) and piggyBac (PB). Also, we designed hybrid vectors combining components from both systems to evaluate new constructs developed in our laboratory: SBnew and PBnew.

These systems, SB and PB, were tested for their efficiency in inserting the CAR gene into T cells. SB used PT2, PT3, and PT4 transposons with the SB100x transposase, while PB transposon with the PBase transposase. T cells were isolated, electroporated with plasmid DNA, activated, and expanded. In vitro and in vivo experiments were performed to evaluate CAR expression, memory phenotype, exhaustion markers, tumor burden, and survival rates in mouse models. SB groups showed a consistent increase in CAR expression over 12 days, while PB had high initial expression that declined over time. CAR-T cells modified using both transposon systems improved survival in mice, with PB demonstrating the lowest tumor burden and highest survival rates. We therefore tested the newly developed constructs to better understand the factors behind PB's higher efficiency, given that the same CAR dose was administered. In vivo testing of CAR-T cells modified with SBnew and PBnew—a hybrid version—showed that SBnew performed better than the original SB, while PBnew did not show improvements compared to PB. We conclude that transposon-based vectors can efficiently mediate CAR gene insertion, with PB proving more effective in reducing tumor burden and improving survival.

Moreover, our results suggest that specific regions from the PB construct may enhance the efficiency of the SB system in CAR-T cells context. This advancement could significantly broaden patient access to CAR-T cell therapy, as these tools are more cost-effective and less labor-intensive than viral approaches. Keywords: CAR-T cells;immunotherapy;Transposon system.

3170 - Category: CELLULAR IMMUNOLOGY (CE)

Dynamic of regulatory T cells in tissue homeostasis and metastatic colorectal cancer

DE MELO, B.M.S.; BARBOSA, G.A.D.C.; SEVERINO, M.B.; LEANDRO, M.D.O.; DAMASCENO, L.E.A.; CONCEIÇÃO, M.L.P.; CASTRO, M.V.D.S.; CUNHA, T.M.; CUNHA, F.Q.; ALVES-FILHO, J.C.. FMRP USP, RIBEIRAO PRETO - SP - BRASIL.

The immune system utilizes different mechanisms to ensure self-tolerance. Regulatory T cells (T_{regs}) are key players in the maintenance of peripheral tolerance and tissue homeostasis. In the context of cancer, both mouse and human tumor microenvironments (TMEs) are enriched with T_{reg} cells, contributing to an immunosuppressive niche suppressing antitumor immune responses and limiting therapeutic success of immunotherapy. Although generalized T_{reg} cell depletion has proven to be efficacious in most preclinical tumor models, it also induces systemic inflammation. Consequently, strategies to reduce their suppressive capacities or destabilize T_{reg} cells specifically in the TME are attractive targets for cancer immunotherapy. In this direction, our project aims to investigate the immunological mechanisms mediated by T_{regs} in the TME, specifically in metastatic colorectal cancer (mCRC). The liver is the most common site for CRC metastases, and up to 75% of patients with mCRC present liver metastases. Although metastatic disease is the main cause of death in CRC, its molecular basis remains poorly defined, which hinders the development of therapeutic strategies. By using complementary multimodal high-throughput and high-dimensional single-cell technologies in conjunction with data-driven computational tools, we identified T_{regs} enriched and engaged with an activation profile, in different subtypes of human CRC, compared with healthy individuals. Furthermore, we identified more than ten subpopulations of Tregs in the steady state, in different tissues, including colon and liver, though multiparametric flow cytometry technologies. We are currently employing different preclinical models of mCRC in mice to deep comprehend the role of T_{regs} in this context. Understanding the dynamic and profile of T_{regs} in this microenvironment may help in the development of new pharmacological strategies to improve the antitumor immune response and increase the survival rate of these patients. Keywords: Regulatory T cells;Homeostasis;Metastatic colorectal cancer.

3171 - Category: CELLULAR IMMUNOLOGY (CE)

Inhibition of BRD4 attenuates features associated with T cell exhaustion and potentiates adoptive T cell-based therapies against tumors

PEREIRA, J.¹; CARVALHO, M.L.R.¹; CAMBUI, R.¹; ARAUJO, E.V.D.O.¹; GUSMAO, A.F.¹; ELIAS, B.D.D.S.¹; PIERRE, M.L.M.¹; DELECHIAVE, D.¹; ABDO, L.D.M.²; CORREIA, E.M.²; LEMOS, M.M.M.¹; BATISTA, M.V.¹; ZEQUI, S.D.C.¹; RINCK JUNIOR, J.A.¹; BEZERRA, S.M.¹; BONAMINO, M.H.²; DE LIMA, V.C.C.¹; KINKER, G.S.¹; CABRAL-PICCIN, M.P.¹; MEDINA, T.D.S.³. 1. A.C. CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 2. INCA - INSTITUTO NACIONAL DE CÂNCER, SÃO PAULO - SP - BRASIL; 3. UNIVERSITY OF SOUTHERN DENMARK, SDU, ODENSE - DINAMARCA.

T lymphocytes play a crucial role in recognizing and eliminating tumor cells, making them ideal candidates for adoptive cell therapies (ACT) against tumors, whether using tumor-infiltrating lymphocytes (TILs), recently approved by the FDA for clinical use in patients with metastatic melanoma, or CAR-T cells, which are used against specific types of hematologic malignancies. However, continuous antigen exposure and the immunosuppressive tumor microenvironment lead to T cell exhaustion, characterized by progressive functional loss associated with epigenetic alterations. We therefore evaluated the impact of different epigenetic inhibitors in order to identify a potent modulator capable of improving T cell functionality in the context of ACT. First, we tested different inhibitors in T cells from healthy donors and observed that the BRD4 inhibitor JQ1 presented a consistent modulatory effect, increasing cell viability while reducing co-expression of inhibitory receptors (PD-1, LAG-3 and TIM-3). Then we tested JQ1 on different ACT modalities, such as anti-CD19 CAR-T cells and TILs from clear cell renal cell carcinoma patients. JQ1 effectively reduced the expression of exhaustion markers in patient-derived TILs and also led to significantly higher numbers of viable cells. The reduced expression of exhaustion markers was also observed in CAR-T cells produced in the presence of JQ1, even after 21 days of culture or in conditions where CAR-T cells were exposed to TGF- β . Furthermore, CAR-T cells treated with JQ1 presented enhanced antitumor activity against the B-ALL cell lineage in vitro. These findings suggest that BRD4 inhibition effectively reduces dysfunction-associated phenotypic markers in T lymphocytes. This approach represents a promising strategy to overcome long-term T cell exhaustion in ACT, potentially enhancing the efficacy of these therapies in both solid tumors and hematological malignancies. Keywords: Adoptive Cell Therapy;Epigenetic Modulation;T lymphocytes.

3189 - Category: CELLULAR IMMUNOLOGY (CE)

Prostaglandin E2 as a potential modulator of the glycolytic pathway during efferocytosis of apoptotic tumor cells by dendritic cells

FERNANDES, L.M.; OLIVEIRA, K.C.; RAIMUNDO, B.V.B.; DE MEDEIROS, A.I.. SCHOOL OF PHARMACEUTICAL SCIENCES - UNESP, ARARAQUARA - SP - BRASIL.

Prostaglandin E2 (PGE2) is a lipid mediator produced in various cellular processes, including the phagocytosis of apoptotic cells (ACs) by phagocytes, such as dendritic cells (DCs), a process known as efferocytosis. This prostanoid has been extensively studied as a mediator that interferes with the tumor microenvironment, potentially promoting the suppression of immune cells. Furthermore, radiotherapy and chemotherapy can induce the apoptosis of tumor cells, and the clearance of these cells leads to the production of anti-inflammatory mediators that affect tumor immunity. Previous data from our group demonstrated that the efferocytosis of glioblastoma ACs (GBM-ACs) upregulated glycolysis-related genes such as *Slc2a1*, *Hk2*, and *Phd3* in DCs, as well as lactate production. Moreover, after 18 hours, the efferocytosis of GBM-ACs downregulates MHC-II and CCR7 while increasing PDL-1 expression and production of IL-6, IL-10, TGF- β , and PGE2, thereby establishing a tolerogenic immune profile. Recent studies indicate that PGE2 regulates the activation of macrophages through metabolism. However, the impact of PGE2 on DC metabolism during efferocytosis remains poorly explored. In this study, we hypothesize that PGE2 may contribute to the acquisition of a tolerogenic profile in DCs after efferocytosis by activating the glycolytic pathway and stabilizing HIF-1 α . To test this hypothesis, bone marrow-derived dendritic cells (BMDCs) from wild-type and HIF-1 α ^{-/-} mice will be co-cultured with GBM-ACs at a 1:3 ratio in the presence of a COX-inhibitor. After the induction of efferocytosis, BMDCs surface molecules will be assessed by flow cytometry, ELISA will quantify the production of mediators, and the metabolic profile will be analyzed by RT-qPCR, Seahorse assays and Western blot, focusing on the activation of the glycolytic pathway and the stabilization of HIF-1 α . This study might contribute to our understanding of the role of PGE2 as a modulator of metabolism and cell activation. Keywords: Efferocytosis;Immunometabolism;Prostaglandin E2.

3195 - Category: CELLULAR IMMUNOLOGY (CE)

Axl signaling worsens the inflammatory response and cellular damage in pulmonary macrophages during COPD

MARINS, J.D.G.¹; MENDES, C.M.¹; NASCIMENTO, J.G.²; SANTOS, J.M.³; DE SÁ, M.C.M.D.M.A.¹; PIMENTA, A.J.⁴; DE LIMA, J.V.C.S.⁵; BRANDÃO, B.P.²; RIBEIRO, J.D.O.²; DA COSTA, A.L.A.²; FERREIRA, J.R.M.¹; PINTO, K.G.²; FILARDY, A.D.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO (UFRRJ), SEROPÉDICA - RJ - BRASIL; 4. UNIVERSIDADE ESTADUAL DO RIO DE JANEIRO (UERJ), RIO DE JANEIRO - RJ - BRASIL; 5. UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO (UNIRIO), RIO DE JANEIRO - RJ - BRASIL.

Smoking is the main cause of chronic obstructive pulmonary disease (COPD), an illness characterized by lung inflammation, cell death, efferocytosis failure, and DNA damage. TAM receptor (Tyro3, Axl, MerTk)-mediated efferocytosis is responsible for initiating anti-inflammatory pathways, reestablishing homeostasis. This study evaluated the role of the Axl receptor in the regulation of lung inflammation in wild-type (WT) and Axl deficient mice (Axl^{-/-}) submitted to a COPD induction protocol that consists of cigarette smoke exposure for three months. We observed 100% survival in all groups and weight loss in COPD-Axl^{-/-} mice on days 15, 30, and 90 when compared to COPD-WT. We analyzed epithelial permeability by protein quantification in the bronchoalveolar lavage (BAL), and we observed an increase in protein concentration in COPD-WT animals when compared to COPD-Axl^{-/-}. We also analyzed immune cell populations present in the BAL using flow cytometry. We found a depletion of resident alveolar macrophages in the COPD groups compared to their controls and an increase in monocyte-derived alveolar macrophages (moAM) and monocyte-derived interstitial macrophages (moIM) in COPD-WT animals when compared to COPD-Axl^{-/-}. We observed a monocyte number increase in both COPD groups compared to their controls, while neutrophils increased significantly in the COPD-WT group compared to the COPD-Axl^{-/-}. BAL cytokine analysis via ELISA revealed a decrease in the levels of CXCL1 and IL-17 in COPD-Axl^{-/-} animals compared to COPD-WT. Lastly, we observed DNA damage induction in moAMs and moIMs via flow cytometry and found both populations in COPD groups had a higher expression of γ H2AX, a DNA damage marker, than their control counterparts. COPD-WT animals had a more pronounced expression of γ H2AX than COPD-Axl^{-/-}. These data suggest that Axl-mediated signaling during COPD amplifies lung inflammation and contributes to the induction of DNA damage in pulmonary macrophages. Keywords: COPD;Alveolar macrophage;Cigarette smoke.

3202 - Category: CELLULAR IMMUNOLOGY (CE)

Antitumor Potential of Bothrops spp. Venoms: A Cytotoxicity Assessment in Leukemia and Lymphoma CellsVALENTIM, C.; PIRES, G.M.M.; FERREIRA JUNIOR, R.S.; MACHADO, M.P.; DA SILVA, T.A.; PUCCA, M.B..
UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO - UNESP, ARARAQUARA - SP - BRASIL.

Snake venoms are complex cocktails of bioactive molecules with remarkable biomedical potential. This study investigated the cytotoxic effects of venoms from seven *Bothrops* species (*B. alternatus*, *B. atrox*, *B. jararaca*, *B. jararacussu*, *B. leucurus*, *B. moojeni* and *B. pauloensis*) on leukemia (Jurkat and Nalm6) and lymphoma (Raji and Daudi) cell lines, aiming to identify candidates for antitumor drug development. Venoms were biochemically characterized by SDS-PAGE and chromatographic techniques, and cytotoxic activity was assessed by the MTT assay. In Jurkat cells, *B. jararacussu* and *B. alternatus* exhibited the strongest effects, inducing ~100% cytotoxicity at 5 µg/mL. In Nalm6 cells, overall sensitivity was lower, but *B. jararacussu* reached ~75% cytotoxicity at 5 µg/mL, whereas *B. jararaca* showed <10% across all concentrations. In lymphoma cells, *B. pauloensis* demonstrated selective potency, with >50% cytotoxicity in Raji cells at 1 µg/mL and >75% in Daudi cells at 5 µg/mL; *B. jararacussu*, *B. alternatus* and *B. moojeni* also induced ~50% cytotoxicity at 5 µg/mL. Importantly, non-cancerous RAW 264.7 macrophages exhibited limited sensitivity, supporting a selective action of certain venoms. The pronounced cytotoxicity of *B. jararacussu* and *B. pauloensis* may be associated with high levels of phospholipases A₂ and metalloproteinases, known to destabilize membranes and induce cell death. In summary, *Bothrops* venoms displayed significant and differential cytotoxicity against leukemia and lymphoma cells, highlighting *B. jararacussu* and *B. pauloensis* as promising sources of bioactive molecules for the development of novel antitumor therapies. Keywords: *Bothrops* venoms; cytotoxicity; leukemia.

3206 - Category: CELLULAR IMMUNOLOGY (CE)

Deciphering the Spatial Landscape of Tregs in Human IBD and Experimental ColitisGARCIA, M.E.M.¹; PACHECO, T.C.F.¹; SILVA, S.L.S.¹; SANTOS, J.S.²; PARRA, R.S.²; FERREIRA, S.D.C.²; FERES, O.³; RAMOS, A.D.S.¹; CUNHA, F.Q.¹; PERES, R.S.¹. 1. DEPARTMENT OF PHARMACOLOGY, RIBEIRAO PRETO MEDICAL SCHOOL, UNIVERSITY OF SAO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. DEPARTMENT OF SURGERY AND ANATOMY, RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO, RIBEIRAO PRETO - SP - BRASIL; 3. DEPARTMENT OF INTERNAL MEDICINE, RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO., RIBEIRAO PRETO - SP - BRASIL.

Inflammatory bowel diseases (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract driven by an exacerbated and dysregulated immune response. Although significant progress has been made in elucidating the molecular and cellular mechanisms underlying IBD pathogenesis, critical gaps remain—particularly regarding the cellular interactions that drive disease progression and contribute to the loss of therapeutic response in patients treated with currently available drugs. Among the immune cell populations involved in IBD, regulatory T cells (Tregs) are of particular interest due to their essential role in maintaining intestinal immune homeostasis and modulating inflammatory responses. This study aims to investigate the spatial organization of Tregs and their interactions with other cell types within the intestinal tissue microenvironment. To this end, intestinal samples from both healthy controls and IBD patients will be analyzed alongside an experimental colitis model induced by the adoptive transfer of naïve T cells into Rag1^{-/-} mice, with or without anti-TNF treatment. Tissue samples will be paraffin-embedded and stained with hematoxylin and eosin (H&E) for histopathological assessment using two standardized scoring systems for both human and murine specimens. Furthermore, spatial transcriptomics will be performed using the Visium HD platform to identify molecular targets and cellular interactions, which will be subsequently validated by immunofluorescence staining. Insights gained from the murine model will be leveraged to interpret and reinforce findings from human samples. Collectively, this work will advance our understanding of the spatial architecture and cellular interactions within the inflamed gut, providing critical insights into disease mechanisms and paving the way for the development of more precise and effective therapeutic strategies for IBD. Keywords: Tregs; Intestinal Inflammation; Cellular interactions.

3210 - Category: CELLULAR IMMUNOLOGY (CE)

EVALUATION OF THE HUMAN RESPONSE TO LdLST: DEVELOPMENT OF A TOOL TO QUANTIFY THE CELLULAR IMMUNE RESPONSE TO *Leishmania* sp.

DA SILVA, L.O.¹; BORBA, P.¹; DE SANTANA, M.B.R.¹; COSTA, T.D.S.¹; TORRES, C.M.L.¹; DE CARVALHO, L.P.¹; SATOSKAR, A.R.²; GANNAVARAM, S.³; NAKHASI, H.L.³; DE CARVALHO FILHO, E.M.¹; DE OLIVEIRA, C.I.¹. 1. FIOCRUZ - BAHIA, SALVADOR - BA - BRASIL; 2. OHIO STATE UNIVERSITY, OHIO - ESTADOS UNIDOS DA AMERICA; 3. U.S. FOOD AND DRUG ADMINISTRATION, SILVER SPRING - ESTADOS UNIDOS DA AMERICA.

In Brazil, *Leishmania braziliensis* is the main etiological agent of localized cutaneous leishmaniasis (LCL), whose clinical manifestations and prognosis are strongly influenced by the host's cellular immune response. The development of tools capable of quantifying this response is essential for evaluating vaccination efforts and identifying biomarkers of protection. We aimed to evaluate the reactivity of peripheral blood mononuclear cells (PBMCs) from individuals naturally exposed to *Leishmania* sp. to LdLST antigen, developed from *L. donovani* and produced under GMP conditions. In this is a cross-sectional, individuals with a history of LCL (healed) (n=16) and with active disease (n=12) were included. Controls consisted of healthy individuals, residing in non-endemic areas (n=7). PBMCs and whole blood were stimulated with LdLST. Preliminary dose-response assays showed that LdLST, at a concentration of 5µg/ml, induces cytokine production (IFN-γ and IL-10) in samples from individuals with active LCL as well as in those with a history of disease. We evaluated the production of cytokines in cell culture supernatants (PBMCs) and plasma (whole blood), upon stimulation with LdLST and with Soluble Leishmania Antigen, prepared from *L. braziliensis*. Cytokine levels were determined by ELISA. LdLST can activate immunocompetent cells and induce recall responses in both isolated cell preparations and unprocessed peripheral blood. Importantly, cytokine production was similar comparing PBMC stimulation with LdLST and SLA, suggesting common antigens in both preparations. Comparing recall responses in individuals with active disease compared to individuals with previous history of disease, the latter showed lower responses (p<0.05). These findings indicate that LdLST is a sensitive tool for the detection and quantification of specific cellular immune responses in individuals previously exposed to a distinct species of *Leishmania* sp. CEP Fiocruz-BA, CAAE: 86870725.0.0000.0040. Keywords: Cutaneous Leishmaniasis; Immune Response; Antigen.

3213 - Category: CELLULAR IMMUNOLOGY (CE)

Phytochemical Screening and Biological Potential of the Aqueous Extract of *Ocimum basilicum*

DO NASCIMENTO, M.D.; DE OLIVEIRA, L.G.; MACEDO, G.C.; ARAGÃO, D.M.D.O.; FABRI, R.L.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Introduction: According to the World Health Organization (WHO), in 2019, non-communicable diseases (NCDs) were responsible for approximately 70% of deaths worldwide. In this context, there has been a growing interest in complementary therapies based on natural products, which has driven scientific research on medicinal plants. Among these, *Ocimum basilicum* (OB), commonly known as basil, stands out. **Objective:** To evaluate the in vitro antioxidant, anti-inflammatory, and cytotoxic activities, as well as to perform the phytochemical characterization of the aqueous extract of OB. **Methods and Results:** Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the β-carotene/linoleic acid system. Cytotoxicity was evaluated through the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) in fibroblast (L929) and macrophage (J774A.1) cell lines. Anti-inflammatory activity was determined by the inhibition of nitric oxide (NO) production and pro-inflammatory cytokines: interleukin-6 (IL-6), interleukin-12 (IL-12), and tumor necrosis factor-α (TNF-α), in stimulated macrophages. Phytochemical screening revealed total phenolic and flavonoid contents of 121.34±8.40 mg tannic acid equivalents/g and 6.17±0.58 mg quercetin equivalents/g of sample, respectively. The extract showed significant antioxidant activity, with low half-maximal inhibitory concentration (IC₅₀) values (DPPH: 2.78±0.61 µg/mL) and protective capacity against β-carotene oxidation. Cell viability remained above 80% in all tested cell lines, in accordance with ISO 10993-5. Anti-inflammatory effects included reduction of NO production (~85%) and cytokine levels (IL-6:~52%; IL-12:~46%; TNF-α:~30%). **Conclusion:** The aqueous extract of OB showed a promising bioactive profile, evidenced by antioxidant and anti-inflammatory activities, low cytotoxicity, and presence of secondary metabolites, suggesting therapeutic potential. Keywords: *Ocimum basilicum*; Antioxidant; Anti-inflammatory.

3222 - Category: CELLULAR IMMUNOLOGY (CE)

Hyaluronan Fragments Modulate Inflammation in Preeclampsia: A Focus on Th17/Treg Balance

VEIGA, M.R.¹; VASQUES, V.R.R.¹; DA SILVA, P.B.²; FRANCO, G.D.O.²; DE OLIVEIRA, L.R.C.¹; PERAÇOLI, J.C.²; PERAÇOLI, M.T.S.¹. 1. INSTITUTE OF BIOSCIENCES, SAO PAULO STATE UNIVERSITY-UNESP, BOTUCATU - SP - BRASIL; 2. BOTUCATU MEDICAL SCHOOL, SAO PAULO STATE UNIVERSITY-UNESP, BOTUCATU - SP - BRASIL.

Pregnant women with preeclampsia (PE) exhibit an exacerbated immune response. This inflammation can be identified by elevated levels of damage-associated molecular patterns (DAMPs), such as hyaluronan (HA). When degraded into low molecular weight fragments, this molecule triggers an inflammatory response, activating immune system cells and leading to increased production of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF). This study aimed to evaluate CD44 expression on CD4⁺ T lymphocytes from pregnant women with PE, before and after stimulation with high molecular weight hyaluronan (hmw-HA) or low molecular weight hyaluronan (lmw-HA). Forty pregnant women, comprising 20 with preeclampsia and 20 normotensive pregnant women were investigated. Plasma samples were separated for TNF, IL-10, HA, and soluble CD44 (sCD44) analyses by ELISA. Determination of CD44 receptor expression, as well as intracytoplasmic transcription factors T-bet (Th1), RORyt (Th17), and Foxp3 (Treg), and cytokines TNF, IL-17, and IL-10, was assessed by flow cytometry. Experiments were conducted after separation of mononuclear cells from peripheral blood (endogenous expression), followed by cell culture with or without hmw-HA and lmw-HA. Pregnant women with PE showed elevated plasma concentrations of hyaluronan and TNF, while sCD44 and IL-10 levels were decreased compared to normotensive pregnancies. The percentage of CD44-expressing cells was higher in lymphocytes expressing T-bet, RORyt, TNF, and IL-17. Stimulation with lmw-HA led to increased T-bet and RORyt and decreased FoxP3 expression in normotensive pregnancies. Conversely, hmw-HA decreased RORyt, IL-17, and TNF expression, while increasing FoxP3 and IL-10 expression in pregnant women with PE. Overall, these findings highlight the significant modulatory role of hmw-HA in rebalancing the Th17/Treg ratio, reducing inflammation, and promoting an anti-inflammatory response in pregnant women with PE. Keywords: preeclampsia;hyaluronan;T cells.

3238 - Category: CELLULAR IMMUNOLOGY (CE)

Axl deficiency skews innate immune responses and worsen clinical outcomes in lung infection

FERREIRA, J.R.M.; MENDES, C.M.; SANTOS, J.M.; MARINS, J.D.G.; NASCIMENTO, J.G.; DE LIMA, J.V.C.S.; DE SÁ, M.C.M.D.M.A.; PIMENTA, A.J.; RIBEIRO, J.D.O.; DA COSTA, A.L.A.; PINTO, K.G.; TODESCHINI, A.R.; FILARDY, A.D.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Pneumonia is the leading infectious cause of death worldwide and is characterized by the accumulation of apoptotic cells in the lungs, which can compromise tissue homeostasis and immune function. Efferocytosis (EF), the process of clearing dying cells, is essential for the resolution of inflammation, but its role during opportunistic bacterial infections such as pneumococcal pneumonia remains poorly understood. This study investigates the function of the receptor Axl, a known mediator of EF, in modulating the immune response against *Streptococcus pneumoniae*. Given the urgent need for alternative therapies due to rising antimicrobial resistance, understanding EF pathways may lead to novel strategies to improve bacterial clearance and clinical outcomes. Wild-type (WT) and Axl-deficient mice were infected intratracheally with *S. pneumoniae*, and immune responses were evaluated in the bronchoalveolar lavage fluid (BALF) at 1-, 2-, and 3-days post-infection (dpi). Axl-deficient mice displayed more severe clinical symptoms, including greater weight loss, hypothermia, higher clinical scores, and reduced spontaneous movement. These mice also exhibited increased bacterial loads in the lungs (3 dpi), early-onset bacteremia (1 dpi), and altered immune cell dynamics, including reduced numbers of alveolar macrophages, impaired recruitment of interstitial and monocyte-derived macrophages, and enhanced infiltration of inflammatory monocytes and neutrophils. Moreover, Axl-deficient mice showed elevated levels of CCL2 and KC chemokines, increased frequencies of apoptotic and necrotic neutrophils, and a proinflammatory cytokine profile marked by higher TNF (3 dpi) and IL-6 (2 dpi). These results indicate that Axl plays a critical role in maintaining immune balance and promoting effective resolution of pneumococcal pneumonia. Keywords: Efferocytosis;Pneumococcal pneumonia;Axl receptor.

3241 - Category: CELLULAR IMMUNOLOGY (CE)

The role of MerTk in modulating streptococcal pneumoniae in mice exposed to cigarette smoke.

SANTOS, J.M.¹; MENDES, C.M.¹; NASCIMENTO, J.G.¹; MARINS, J.D.G.¹; DE SÁ, M.C.M.D.M.A.¹; DE LIMA, J.V.C.S.²; PIMENTA, A.J.³; RIBEIRO, J.D.O.¹; DA COSTA, A.L.A.¹; FERREIRA, J.R.M.¹; PINTO, K.G.¹; FILARDY, A.D.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Smoking is the main triggering factor for the development of Chronic Obstructive Pulmonary Disease (COPD), a condition marked by chronic, progressive inflammation in the lungs, airflow limitation, and increased cell death. This pathological scenario is frequently associated with defects in efferocytosis, the process by which apoptotic cells are cleared by phagocytes. Among the key regulators of efferocytosis are the TAM family receptors (Tyro3, Axl, and MerTk) with MerTk playing a central role in maintaining pulmonary homeostasis. In smokers with COPD, MerTk function is often impaired, contributing to persistent inflammation and tissue damage. This unresolved inflammation also increases susceptibility to infections, especially pneumonia caused by *Streptococcus pneumoniae*. Clearance of this pathogen requires coordinated activity of innate immune cells, particularly macrophages. In this study, we investigated the role of MerTk in immune regulation during pneumococcal pneumonia in a model of cigarette smoke exposure. We performed adoptive transfer of monocyte-derived alveolar macrophages (moAMs) from wild-type mice into WT and MerTk-deficient (MerTk^{-/-}) mice previously exposed to smoke and infected with *S. pneumoniae*. MerTk^{-/-} mice exhibited lower survival, worsened clinical outcomes (greater weight loss, prolonged hypothermia, and higher clinical scores), and increased inflammatory cell infiltration, especially neutrophils and monocytes. A significant reduction in resident alveolar macrophages was observed, suggesting impaired maintenance of this population. While total moAM and monocyte-derived interstitial macrophage (moIM) numbers remained unchanged, moAMs from MerTk^{-/-} mice showed a more pro-inflammatory profile (MHCII⁺), where moIMs exhibited elevated CD206 expression, indicating phenotypic divergence. These results highlight that in the absence of MerTk, moAM transfer exacerbates pulmonary inflammation following smoke exposure and infection. Keywords: Smoking;Streptococcus pneumoniae;MerTk.

3245 - Category: CELLULAR IMMUNOLOGY (CE)

Lymphocytic analysis of alopecia in lupus using innovative high-dimensional flow cytometry methodology

DA SILVA, I.B.¹; PINHEIRO, D.F.¹; PANASCO, M.D.S.¹; GANDINI, M.²; PARISIO, J.M.¹; DE BRITO, J.P.¹; EL-KADI, N.T.¹; BORGES, A.C.¹; GAVAZZONI, M.F.R.¹; SILVA, A.A.¹. 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Alopecia is a manifestation present in approximately 85.2% of patients with lupus, a chronic autoimmune disease with a significant imbalance in the cellular immune response and elevated autoantibody production. Few studies have analyzed the cellular phenotype in lupus, with most using blood cells, which have reported mainly follicular helper T cells, regulatory T cells, and independent T cells. **Aim:** We aimed at standardizing a protocol for purifying single-cell suspensions from scalp tissue from lupus patients. **Methods:** To do so, we recruited a patient with lupus alopecia from the rheumatology and dermatology departments of HUAP/EBSERH-UFF to perform a 6mm scalp biopsy and PBMCs cell purification for extracellular and intracellular staining with anti-CD3-BUV496, -CD45-BUV395, -CD4-R718, -CD45RO-BV480, -CD25-BV421, -CD8-BUV-805, -CD127-BUV563, -TCR-Gamma-Delta-BV650, -CD103-BB700, -CD69-PE-594, -CCR6-AlexaFluor-647, -PD1-BV711, -CCR4-PECY7 and -T-bet-PE, -KI67-PECY5, -FOXP-AlexaFluor-488 antibodies, which then were analyzed on the Symphony A5 flow cytometer. **Results:** We grouped 22 cell subpopulations in the biopsy and 45 in the PBMCs using the UMAP and phenograph algorithm. Through analysis of these clusters using the algorithm cluster explorer, we identified TH1, TH2, TH17, exhausted T CD8 cells, tissue resident cells, among others, and could evaluate the mean intensity of fluorescence of each marker in the clusters. By correlating blood and scalp cells, which have an efficient cell yield above 6.000 cells, we observed that the biopsy contained a majority of TH17 T cells and tissue resident T cells. **Conclusion:** The standardized panel of 16 antibodies allows us to identify lymphocytic subpopulations, contributing to our understanding of the immune response in lupus-associated alopecia, which will possibly further dictate correlations with skin and systemic disease. Keywords: Lupus;Alopecia;High-dimensional flow cytometry.

3248 - Category: CELLULAR IMMUNOLOGY (CE)

THE ROLE OF EXTRACELLULAR VESICLES FROM PLASMA AND ADIPOSE TISSUE DERIVED FROM INDIVIDUALS WITH OBESITY IN THE MODULATION OF PBMC

GAMEIRO, J.¹; JANUZZI, L.L.R.¹; VENERANDO, L.A.F.¹; DIB, P.R.B.¹; LIMA, M.J.T.¹; FERNANDES, M.K.C.²; PERCEGONI, N.¹; CANGUSSU, V.³; SANTOS, M.D.O.²; JANUZZI, G.L.R.¹; HOTTZ, E.D.¹. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 3. HOSPITAL E MATERNIDADE THEREZINHA DE JESUS, JUIZ DE FORA - MG - BRASIL.

Obesity is a chronic multifactorial disease characterized by excessive accumulation or abnormal distribution of body fat, primarily classified by the body mass index (BMI, kg/m²). It is associated with elevated levels of circulating pro-inflammatory cytokines, largely secreted by adipose tissue (AT), which lead to persistent immune system activation. Extracellular vesicles (EVs) are nanostructures enclosed by a lipid bilayer, secreted by various cell types, and play an active role in intercellular communication and immune regulation. This study aimed to evaluate the response of peripheral blood mononuclear cells (PBMCs) from eutrophic (BMI < 25) and obese individuals (BMI > 30) upon exposure to EVs isolated from AT of bariatric patients (n=3), and from plasma of obese (n=6) and eutrophic (n=3) individuals. PBMCs, after 4 hours of isolation, were treated with EVs for 24 or 48 hours at a concentration of 50 µg/mL. AT-derived EVs were isolated using centrifugation and ultracentrifugation protocols, while plasma-derived EVs were obtained using size exclusion chromatography (SEC), which effectively reduced albumin and cholesterol contamination, as verified by cholesterol quantification. EVs were characterized using ZetaSizer, transmission electron microscopy, Dot Blot, BCA assay, and fluorescence microscopy. Plasma EVs from both eutrophic and obese individuals modulated PBMCs toward an anti-inflammatory profile, increasing CD14⁺ and CD14⁺CD16⁺ populations and reducing CD16⁺, CD3⁺, and CD16⁺CD3⁻ (NK) cells, with more pronounced changes induced by obese-derived EVs. IL-1β, CCL-2, and RANTES secretion was reduced at 24h and increased at 48h by obese plasma EVs. In contrast, AT-derived EVs increased TNF-α production at 24h. Overall, EVs from obese individuals induced a pro-inflammatory profile in immune cells. Keywords: obesity;extracelullar vesicles;inflammation.

3254 - Category: CELLULAR IMMUNOLOGY (CE)

Influence of antigen quantity on the generation of CD8⁺ TRM precursors during the acute phase of genital HSV-2 infection

HAUBERT, A.F.¹; PELLEZ, M.C.M.¹; LINS, M.²; GONÇALVES, J.V.P.¹; GASSEN, R.B.¹; ROMÃO, P.R.T.¹; RODRIGUES JUNIOR, L.C.¹. 1. UFCSPA, PORTO ALEGRE - RS - BRASIL; 2. UFMT, CUIABÁ - MT - BRASIL.

Introduction: Herpes Simplex Virus-2 (HSV-2) is a leading cause of recurrent genital ulcers, and its control relies heavily on CD8⁺ T cells. Among these, tissue-resident memory CD8⁺ T cells (CD8⁺ TRMs) persist in peripheral tissues, such as the genital mucosa, acting as frontline sentinels capable of mounting rapid immune responses. The differentiation and maintenance of CD8⁺ TRMs are regulated by local antigen availability, inflammation, and tissue-specific factors. In the context of HSV-2 infection, the presence of cognate antigen plays a critical role in driving CD8⁺ TRM formation. During primary infection, patients frequently receive the antiviral to reduce viral load, this may diminish antigen exposure and thereby impair the development of TRM populations.

Objective: To investigate whether varying inoculum levels of HSV-2 during primary genital mucosal infection influence the generation of CD8⁺ TRM precursors.

Methods: Female C57BL/6 mice were intravaginally infected with low (7.5×10^3 PFU/ml), medium (1.5×10^4 PFU/ml), or high (3×10^4 PFU/ml) doses of HSV-2. Fourteen d.p.i, genital tissues and spleens were harvested for lymphocyte isolation. Flow cytometry was used to identify CD8⁺ TRM cells, characterized as CD3⁺CD8⁺CD44⁺CD69⁺CD103⁺CD49a⁺. Antigen specificity of CD8⁺ T cells was evaluated via intracellular IFN-γ production following in vitro stimulation with the HSV-2 peptide SSIEFARL.

Results: Higher HSV-2 doses led to a more robust accumulation of CD8⁺ T cells in the genital mucosa, with increased frequencies of CD8⁺CD44⁺ effector cells expressing the TRM markers CD69, CD103, and CD49a. Upon stimulation with the SSIEFARL peptide, these cells produced IFN-γ, confirming antigen specificity.

Conclusion: Antigen availability during primary HSV-2 infection influences the generation of effector CD8⁺ T cells and the establishment of TRM cells in the genital mucosa. Higher viral loads during the priming phase favor the development of a more robust TRM population.

Keywords: TRM Cells;HSV-2 Infection;Mucosal Immunity.

3254 - Category: CELLULAR IMMUNOLOGY (CE)

Axl receptor activation emerges as a key regulator of alveolar macrophage immune response during *Streptococcus pneumoniae* infection

MENDES, C.M.¹; SANTOS, J.M.¹; NASCIMENTO, J.G.¹; MARINS, J.D.G.¹; DE SÁ, M.C.M.D.M.A.¹; DE LIMA, J.V.C.S.²; PIMENTA, A.J.³; RIBEIRO, J.D.O.¹; DA COSTA, A.L.A.¹; PINTO, K.G.¹; FERREIRA, J.R.M.¹; FILARDY, A.D.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO (UNIRIO), RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE ESTADUAL DO RIO DE JANEIRO (UERJ), RIO DE JANEIRO - RJ - BRASIL.

Streptococcus pneumoniae (Sp) is an opportunistic pathogen linked to severe respiratory infections, such as pneumonia, particularly in immunocompromised individuals. Alveolar macrophages (AMs) constitute the first line of defense in the lungs, responsible for eliminating pathogens and apoptotic cells through efferocytosis - a critical process in the regulation of inflammation. The Axl receptor, a member of the TAM family, plays a key role in this pathway by promoting the resolution of inflammation and supporting cell survival. However, during acute bacterial infections like those caused by Sp, the role of Axl in modulating AM responses remains poorly understood. In this study, we investigated, *in vitro*, how the absence of Axl affects phagocytosis, intracellular bacterial killing, and AM viability during Sp infection. AMs were isolated via bronchoalveolar lavage from wild-type (WT) C57BL/6 mice and Axl-deficient (Axl^{-/-}) mice (CEUA license n° 057/22). Cells were plated (1×10⁵ per well) and subsequently infected with Sp (MOI 1:300). Phagocytic activity and bacterial killing were assessed by plating cell lysates on sheep blood agar, while cell viability was evaluated by flow cytometry. Axl^{-/-} AMs showed a significantly reduced phagocytic capacity and decreased efficiency in bacterial clearance compared to WT AMs. Flow cytometry analysis further revealed a marked increase in late apoptosis and necrosis in Axl^{-/-} AMs following Sp infection. These findings suggest that Axl-mediated signaling enhances bacterial clearance and supports AM survival, highlighting a protective role for this receptor during Sp infection. Keywords: Axl receptor; *Streptococcus pneumoniae*; Alveolar macrophages.

3258 - Category: CELLULAR IMMUNOLOGY (CE)

Impact of HIF-1α in mediating the tolerogenic profile of dendritic cells during efferocytosis of glioblastoma apoptotic cells

DOS REIS, R.H.; OLIVEIRA, K.C.; RAIMUNDO, B.V.B.; DE MEDEIROS, A.I.. UNESP - CAMPUS ARARAQUARA, ARARAQUARA - SP - BRASIL.

Dendritic cells and macrophages are the main phagocytes involved in the clearance of apoptotic bodies. This process, known as efferocytosis, occurs under both homeostatic and pathological conditions. Dendritic cells play a crucial role in the antitumor response by presenting tumor cell antigens to T cells. The phagocytosis of non-immunogenic dead cells, induced by radiation or some chemotherapeutic agents, contributes to the generation of a suppressive tumor microenvironment, compromising the effectiveness of the antitumor response. Previous studies have shown that blocking the efferocytosis of apoptotic tumor cells alters the immunogenic profile of dendritic cells and impairs tumor progression. Recent findings from our group demonstrate that efferocytosis of glioblastoma apoptotic cells (GBM-ACs) by bone marrow-derived dendritic cells (BMDCs) promotes a tolerogenic profile, characterized by the production of IL-10, TGF-β, and IL-6, as well as the down-regulation of MHC-II and CCR7, and up-regulation of PD-L1. In addition, we observed glycolysis-related gene expression, such as *Phd3*, a coactivator of HIF-1α, and *Glut1*, as well as an increase in lactate production. Therefore, we hypothesize that efferocytosis of GBM-ACs leads to stabilization of HIF-1α, which supports the glycolytic pathway and promotes the development of a tolerogenic profile in dendritic cells. To test the hypothesis, BMDCs from C57BL/6 and HIF-1α KO (HIF-1α^{fl/fl}/fLyzMCre) mice will be co-cultured with GBM-ACs and determine i) the impact of GBM-AC efferocytosis on the activation and stabilization of HIF-1α in dendritic cells; ii) whether the deficiency of HIF-1α impacts the induction of the tolerogenic dendritic cells and metabolic profile, focusing on the glycolytic pathway. These results may provide new insights into the interaction between cellular metabolism and immunosuppression within the tumor microenvironment. Keywords: Dendritic cell; Efferocytosis; HIF-1α.

3261 - Category: CELLULAR IMMUNOLOGY (CE)

Axl-dependent regulation of mucosal immunity during bacterial colitis

DE SÁ, M.C.M.D.M.A.; NASCIMENTO, J.G.; DE LIMA, J.V.C.S.; DA COSTA, A.L.A.; RIBEIRO, J.D.O.; MENDES, C.M.; FILARDY, A.D.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

The intestinal mucosa undergoes constant apoptosis, requiring efficient efferocytosis by phagocytes to prevent inflammation. TAM receptors (Tyro-3, Axl, MerTK) mediate this process and promote immune resolution via anti-inflammatory pathways. In colitis, macrophage efferocytosis leads to pro-resolving mediator release, limiting disease severity. Impairment in this process increases susceptibility to intestinal inflammation. Current treatments rely on antibiotics, raising concerns about antimicrobial resistance. Thus, identifying new therapeutic targets, such as TAM receptors, for bacterial colitis offers a promising strategy to improve disease management and reduce reliance on conventional antibiotics. In this study, we investigated the role of Axl-mediated efferocytosis in mice with bacterial colitis. Wild-type C57BL/6J mice (WT) and Axl-deficient mice (Axl KO) were used and were gavaged with PBS or 10^8 CFU/mL of *Citrobacter rodentium* (strain ICC100). After 14 days, the colons were collected for cytokine quantification by ELISA. Lamina propria leukocytes were isolated from the colon for flow cytometry. To assess intestinal permeability, spleen and fecal homogenates were plated on LB agar for colony counting. All infected groups showed greater loss of body weight compared to the PBS-treated groups, regardless of genotype. In the spleen, a higher bacterial colony count was detected in the infected WT group compared to the infected Axl KO group, suggesting altered intestinal permeability and bacterial translocation from the gut to the spleen. Finally, analysis of lamina propria cells revealed an increase in the percentage of colonic macrophages and monocytes in the infected Axl KO group compared to the PBS group. This value was higher than in both WT groups. Our data suggest that the Axl receptor is important in regulating colonic macrophage and monocyte populations, which are essential for patrolling and maintaining gut homeostasis during the development of colitis. Keywords: Receptors TAM;Efferocytosis;Colitis.

3262 - Category: CELLULAR IMMUNOLOGY (CE)

From Blood Smears to Welfare Insights: Developing an Innovative Scoring System Based on Leukocyte Morphology for Animal Welfare Assessment

RIBEIRO, L.R.R.¹; SANTOS, R.M.²; MOLENTO, C.F.M.¹. 1. UFPR, CURITIBA - PR - BRASIL; 2. EBPÓS, CAMPO GRANDE - MS - BRASIL.

Leukocytes are promising indicators of immune status and animal welfare, as they sensitively reflect an organism's responses to environmental, health-related and emotional challenges. Despite the frequent use of leukocyte counts and heterophil-to-lymphocyte or neutrophil-to-lymphocyte ratios, there is still a lack of standardized morphological criteria that allow for a consistent and detailed interpretation of leukocyte alterations across species and production systems. This study proposed to: (1) describe the structure and function of heterophils in broiler chickens and neutrophils in beef cattle; (2) develop a standardized cell scoring system to assess welfare conditions based on leukocyte morphology; and (3) create an illustrated guide detailing morphological criteria, scoring scales, and the classification of heterophils, neutrophils and lymphocytes in both species. The classification considered alterations such as cytoplasmic granulation, nuclear segmentation, vacuolization, chromatin density, and the presence of immature forms. In broiler chickens, heterophil alterations were scored from 0 to 4 and lymphocyte changes from 0 to 1. In cattle, neutrophils were scored from 0 to 4 and lymphocytes from 0 to 1. These scores were graded by severity and interpreted in correlation with field-based welfare assessments, as previously reported. This innovative system transforms routine blood smears into meaningful welfare insights, integrating leukocyte morphology with an accessible and ethically grounded approach, and highlights its potential as a sensitive tool for early welfare assessment in farm animals. Keywords: heterophil;neutrophil;leukocyte.

3272 - Category: CELLULAR IMMUNOLOGY (CE)

Inflammatory Profile and B Cell Dysfunction as Predictors of Readmission and Death in People Living with HIV/AIDS

FERREIRA, S.C.¹; XAVIER, T.D.S.¹; DA SILVA, A.Q.P.²; PRAXEDES, I.R.¹; LACO, A.C.S.¹; PINHEIRO, R.D.S.¹; NOGUEIRA, P.A.¹; FREIRE, M.³; FERREIRA, L.C.D.L.⁴; CHAVES, Y.O.¹. 1. ILMD/FIOCRUZ AMAZÔNIA, MANAUS - AM - BRASIL; 2. PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA BÁSICA E APLICADA, MANAUS - AM - BRASIL; 3. FCECON, MANAUS - AM - BRASIL; 4. FMT-HVD, MANAUS - AM - BRASIL.

Human Immunodeficiency Virus (HIV) infection is associated with dysfunction of several immune cells, including B lymphocytes, although the underlying mechanisms remain unclear. This study aimed to investigate the relationship between B cell subpopulations, inflammatory biomarkers, and clinical outcomes, specifically hospital readmission and mortality, among people living with HIV/AIDS (PLWHA) hospitalized at a tertiary referral center in the state of Amazonas, Brazil. Blood samples were collected from 56 PLWHA within 24 hours of hospital admission. Participants were categorized into four clinical groups: discharge without readmission, readmission, readmission followed by death, and in-hospital death. Cytokines IL-8, IL-1 β , IL-6, IL-10, TNF, and IL-12p70 were quantified using flow cytometry (CBA), while plasma levels of soluble CD14 (sCD14) and IgM were measured by ELISA. Immunophenotyping of peripheral blood mononuclear cells (PBMCs) was performed to characterize B cell subpopulations based on CD19, CD27, CD70 expression, and intracellular IgM production. A male predominance was observed across all groups, along with low CD4+ T cell counts and high viral loads at admission. Inflammatory cytokines, particularly IL-6, IL-8, and IL-10, were significantly elevated in patients who progressed to death. Higher sCD14 and IgM levels were especially prominent among patients readmitted and later deceased, indicating persistent immune activation. Altered B cell subpopulations were observed in groups with worse outcomes, correlating with an intensified inflammatory profile. These findings suggest that disturbances in inflammatory markers and B cell function may help identify PLWHA at increased risk of readmission and mortality, particularly i. Keywords: .

3273 - Category: CELLULAR IMMUNOLOGY (CE)

Platelet-lymphocyte interactions in Long COVID

OLIVEIRA, R.T.R.¹; DE PAULA, M.M.L.¹; DOS SANTOS, J.P.R.S.¹; GOMES, M.T.¹; DIB, P.R.B.¹; SHIGAEFF, N.¹; TOSCANO, E.C.D.B.¹; CARVALHO, W.A.²; BOZZA, P.T.³; WEYRICH, A.⁴; HOTTZ, E.D.¹. 1. UFJF, JUIZ DE FORA - MG - BRASIL; 2. EMBRAPA GADO DE LEITE, JUIZ DE FORA - MG - BRASIL; 3. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 4. OKLAHOMA MEDICAL RESEARCH FOUNDATION, OKLAHOMA - ESTADOS UNIDOS DA AMERICA.

Long COVID is a chronic condition that may develop after SARS-CoV-2 infection. Symptoms range from mild to severe, including respiratory, neurological and thrombotic complications, and lasting weeks, months or years. Long-term effects could result in disabilities, and to understand the mechanisms underlying the development of long COVID is necessary. Studies demonstrate that platelets can modulate lymphocyte function, acting as important regulators of the immune system. The aim of this study is to analyze platelet-lymphocyte interaction in Long COVID patients with cognitive impairment. Our cohort included individuals who had mild acute COVID with a positive test for SARS-CoV-2 in the last 12 months, classified into Long COVID (n=16) or Recovered (n=9) after application of a cognitive tests. Blood samples were collected and lymphocytes subsets and platelet-lymphocytes interactions were analyzed by flow cytometry. B cells were classified as naïve (CD38-CD27-), memory (CD38-CD27+) or immature transitional cells (CD38+CD27+-). CD4+ and CD8+ T-cell subsets were quantified as naïve (CD62L+CD45RA+), central memory (CD62L+CD45RA-), effector memory (CD62L-CD45RA-) or terminally differentiated effector memory (TEMRA) (CD62L-CD45RA-). Platelet-lymphocyte interactions were analyzed by CD41 expression. The total level of B cells (CD19+CD20+-) showed a tendency to decrease (p=0,06) in Long COVID patients, besides no differences were observed between subpopulation frequency and aggregation with platelets. Long COVID patients showed a similar percentage of TCD4+ and TCD8+ subsets compared to the recovered group, however, platelet-T cell aggregate formation was higher in TCD4+ (p=0,02) and TCD8+ (p=0,04) naïve subset. As platelets are capable of lymphocyte modulation through direct cell-cell contact, the higher aggregation observed in Long COVID patients could impact lymphocytic response and disease outcomes. More studies are necessary to confirm this hypothesis. Keywords: Long COVID;Platelet-lymphocyte interaction;Lymphocyte subsets.

3280 - Category: CELLULAR IMMUNOLOGY (CE)

The Impact of Vitamin D Deficiency on Thromboinflammation in Individuals with Obesity

FERNANDES, M.K.C.¹; DIB, P.R.B.¹; MANCINI, M.C.B.¹; VENERANDO, L.A.F.¹; DE SOUZA, V.P.¹; DE LIMA, M.F.C.¹; CARVALHO, W.A.²; PERCEGONI, N.¹; GAMEIRO, J.¹; HOTTZ, E.D.¹. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. EMBRAPA GADO DE LEITE, JUIZ DE FORA - MG - BRASIL.

Obesity is a chronic low-grade inflammatory condition associated with increased thrombotic risk. Vitamin D deficiency is frequently observed as a factor that aggravates the inflammatory and procoagulant profile in obese individuals. Our aim was to investigate the influence of vitamin D on tissue factor (TF) and inflammatory mediator secretion by monocytes in different pro-inflammatory contexts related to obesity. We analyzed TF expression in monocytes, neutrophils and plasma, as well as plasma thromboinflammatory markers, correlating these findings with vitamin D levels. We also evaluated the release and cellular origin of pro-coagulant extracellular vesicles (EVs) (AnnV⁺TF⁺) using flow cytometry. Plasma exchange experiments exposed whole blood from healthy donors to plasma from obese or eutrophic individuals, with or without vitamin D deficiency. Finally, inflammatory stimuli (lipopolysaccharide (LPS) and leptin) were applied in vitro to monocytes from obese and eutrophic subjects, in the presence or absence of 1,25(OH)₂D₃ and secretion of TF, IL-1 β , IL-8 and TNF- α was measured by ELISA. The study was approved by the ethics committee 5.736.117. Results demonstrated that individuals with obesity and vitamin D insufficiency had higher TF expression in monocytes and increased release of monocyte-derived procoagulant EVs. Moreover, plasma from obese individuals with vitamin D deficiency induced higher TF expression in monocytes from healthy donors compared to plasma from eutrophic individuals, suggesting a role for soluble procoagulant mediators. The effects of inflammatory stimuli on TF, IL-1 β , IL-8 and TNF- α secretion by monocytes from obese individuals stimulated with LPS or leptin were reduced by vitamin D treatment. These findings support that the obesity inflammatory microenvironment, exacerbated by vitamin D deficiency, contributes to thromboinflammatory pathways and that vitamin D may modulate monocyte procoagulant responses.

Keywords: Obesity;Immunometabolism;Vitamin D.

3283 - Category: CELLULAR IMMUNOLOGY (CE)

IL-10 Modulates Metabolism and Dendritic Cell Function During Efferocytosis of Staphylococcus aureus-Infected Cells

RAGONESE, F.; PENTEADO, L.D.A.; OLIVEIRA, K.C.; RAIMUNDO, B.V.B.; DE MEDEIROS, A.I.. SCHOOL OF PHARMACEUTICAL SCIENCES - UNESP, ARARAQUARA - SP - BRASIL.

During Staphylococcus aureus methicillin-resistant (MRSA) skin infection, neutrophils engulf the bacteria and then undergo apoptosis, promoting the accumulation of MRSA-infected apoptotic cells (MRSA-iAC). Previous studies have shown that efferocytosis of apoptotic cells (AC) induces metabolic reprogramming in macrophages. However, the impact of iAC-efferocytosis on cell metabolism and its regulation of DC function remains to be investigated. Our group demonstrated that MRSA-iAC efferocytosis reduces mitochondrial respiration and causes mitochondrial dysfunction in DC after 6h. However, after 18h, we observed partial restoration of mitochondrial dysfunction and an increase in IL-10 production. It has been shown that IL-10 regulates mitochondrial integrity and glycolysis in macrophages in the presence of LPS. Therefore, we hypothesized that IL-10 may play a role in restoring mitochondrial function and modulating DC activation during the efferocytosis of MRSA-iAC. BMDC from WT and IL-10-deficient (KO) mice were co-cultured with MRSA-iAC, and the role of IL-10 in metabolism and DC activation was evaluated by Flow cytometry, ELISA and RT-PCR. MRSA-iAC efferocytosis increased MHC-II, CD86, and PDL-1, while IL-10 deficiency reduced only MHC-II and CD86. MRSA-iAC also enhanced IL-1 β , IL-6, TNF- α , and IL-10 production; IL-10 absence further increased TNF- α and IL-1 β but did not affect IL-6 or nitrite. Also, IL-10-deficient DCs did not affect mitochondrial mass and had a minimal impact on mitochondrial membrane potential; however, after 18h, the deficiency of IL-10 showed an accumulation of dysfunctional mitochondria during MRSA-iAC efferocytosis, compared to WT. On the other hand, the absence of IL-10 increased Glut1 expression during MRSA-iAC efferocytosis, compared to control and DC+AC. Taken together, our findings suggest that IL-10 downregulates DC activation, suppressing the glycolytic pathway, and also impairs the accumulation of dysfunctional mitochondrial. Keywords: Dendritic Cells; Immunometabolism;IL-10.

3295 - Category: CELLULAR IMMUNOLOGY (CE)

Effects of nicotinamide supplementation on macrophage susceptibility to cell deathMESSNER, F.; BOZZA, M.T.; NETO, H.A.D.P.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Resistance and tolerance are two complimentary mechanisms of host immune responses. Resistance reduces pathogen load, while tolerance mitigates tissue damage caused by both pathogen and immune system.

Metabolic stresses, such as physical exercise, caloric restriction or intermittent fasting, cause energy depletion and increase cellular NAD⁺/NADH ratio. NAD⁺ is a substrate for SIRT6 and poly (ADP-ribose) polymerases (PARPs), enzymes involved in fundamental processes like regulation of gene expression and DNA damage repair. Low-intensity, long-term stress leads to cellular adaptations that turn cells more resilient to subsequent high-intensity stress, a process called hormesis. We hypothesized that preconditioning cells with metabolic stressful stimuli could induce cellular adaptations to boost tissue tolerance, characterized by resistance to cell death.

We used bone-marrow derived macrophages (BMDMs) as a cell model, since macrophages are capable of promoting or resolving inflammation and tissue damage. To mimic metabolic stress in vitro, we treated BMDMs with the NAD⁺ precursor, nicotinamide (NAM), to increase NAD⁺/NADH ratio. After preconditioning with NAM, cells were challenged with doxorubicin, to induce apoptosis.

Our results show that, at higher concentrations, NAM increases doxorubicin-induced apoptosis, as seen by MTT and flow-cytometry. However, at lower concentrations, NAM was protective. We then analyzed if NAM would differently affect doxorubicin-induced apoptosis in polarized macrophages. BMDMs were polarized with TNF α and IFN γ (for M1) or IL-4 (for M2). Polarization increased the expression of M1 and M2 markers (MHC II or CD206, respectively) as expected. NAM-increased apoptosis was reproduced in M1 but not M2 macrophages. This suggests that high NAD⁺/NADH ratio may spare M2 macrophages to favor resolution of inflammation and tissue regeneration. Our findings highlight metabolic preconditioning as a tunable strategy to enhance tissue tolerance. Keywords: Preconditioning; Macrophages; Apoptosis.

3302 - Category: CELLULAR IMMUNOLOGY (CE)

Platelet Activation and Immune Modulation in ObesityMANCINI, M.C.B.; FERNANDES, M.K.C.; DIB, P.R.B.; DE SOUZA, V.P.; DE LIMA, M.F.C.; PERCEGONI, N.; GAMEIRO, J.; HOTTZ, E.D.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Obesity is characterized by chronic low-grade inflammation associated with a prothrombotic state involving platelet activation and hyperresponsiveness. Emerging evidence implicates platelets in modulating immune responses, particularly in inflammatory settings. Studies have shown that they can express antigen presentation molecules and secrete cytokines and chemokines, indicating a potential role in immune regulation in obesity. However, the mechanisms of platelet activation and immune modulation in obesity remain poorly understood. Our hypothesis is that platelet activation in obesity contributes to thromboinflammation and immune modulation. To investigate this, platelets were isolated from obese and eutrophic individuals and analyzed by flow cytometry and ELISA for platelet activation and antigen presentation markers. The study was approved by the ethics committee 5.736.117. Our results showed that, compared to controls, a higher percentage of platelets from obese patients expressed CD63, a dense granule marker, CD62P, a α -granule marker, and the activated conformation of α IIb β 3 integrin, but not Tissue Factor (TF). No differences were observed in baseline expression of HLA-ABC, CD40, CD80, CD86, or PD-L1. To assess hyperresponsiveness, platelets were stimulated with optimal and suboptimal thrombin concentrations and analyzed by flow cytometry and ELISA with the same markers. Furthermore, platelets from obese patients were hyperresponsive to thrombin with increased surface expression of CD63 and PAC-1, and a trend toward upregulated levels of CCL5. Additionally, increased surface expression of platelet CD80, a costimulatory molecule, was observed under thrombin stimulation. Stimulus-dependent expression suggests a context-dependent immunomodulatory role in obesity. These data show that obese individuals exhibit increased platelet activation and hyperresponsiveness, potentially linked to the regulated surface expression of costimulatory molecules. Keywords: Obesity; Platelets; Hyperresponsiveness.

3305 - Category: CELLULAR IMMUNOLOGY (CE)

Morphological Alterations in Hepatocytes Increase Migratory Impedance of Neutrophils Toward Sites of Sterile Injury.

RAMOS, G.P.; JÚNIOR, W.F.D.S.; CORRADI, P.S.; COELHO, J.; OLIVEIRA, H.M.D.C.; PEDROSA, M.L.M.P.; ANTUNES, M.M.; DE PAULA, C.; MENEZES, G.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Introduction: The liver vasculature is uniquely complex, with sinusoids forming tortuous paths physically confined by hepatocytes. Volume alterations in hepatocytes - due to cytoplasmic changes such as fat accumulation or parenchymal remodeling like fibrosis - can narrow sinusoidal caliber, disrupting blood and leukocyte flow. We previously showed that neutrophils use the intravascular route to reach necrotic areas and support tissue repair. Whether impaired neutrophil migration contributes to defective liver healing in metabolic diseases remains unclear.

Methods and Results: We investigated neutrophil migratory behavior using a murine model of diet-induced steatofibrosis that mimics metabolic liver disease. C57BL/6 WT and LysM-eGFP mice were fed either a control or methionine-choline-deficient (MCD) diet for 8 weeks. We combined this model with high-definition intravital confocal microscopy to image neutrophil swarming in real time and track their intravascular migration toward focal necrosis. Time-lapse movies were analyzed offline using Volocity 6.3, quantifying parameters such as velocity, acceleration, path, and displacement. Systemic and immunologic responses were assessed by liver enzymes, cytokines, and gene expression.

Conclusion: Liver steatosis caused significant alterations in hepatic hemodynamics, reducing blood flow and distorting sinusoidal architecture. In controls, optimal perfusion supported rapid, directional neutrophil migration. In steatotic livers, neutrophils failed to efficiently reach necrosis, showing erratic, ineffective paths and impaired sensing of migrational cues. We propose that mechanical disruption of migratory routes may underlie defective immune-mediated repair in metabolic liver disease. Financial support: FAPEMIG, CAPES, CNPq. **Keywords:** Neutrophils; Liver Fibrosis; Hepatitis.

3306 - Category: CELLULAR IMMUNOLOGY (CE)

The Role of the P2X7 Receptor in Periodontitis-Associated Oral Dysbiosis and its Impact on Ulcerative Colitis

DA CRUZ, L.D.O.¹; DA COSTA, A.L.A.²; SOARES, M.A.³; CÔRTEZ, T.N.¹; BAVIER, A.C.D.¹; LOPES, G.L.D.M.M.⁴; COLOMBO, A.P.V.⁵; FILARDY, A.D.⁵; DE SOUZA, H.S.P.²; SAVIO, L.E.B.¹; COUTINHO-SILVA, R.¹. 1. IBCCF, UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. ICB, UFRJ, RIO DE JANEIRO - RJ - BRASIL; 4. UNIGRANRIO, RIO DE JANEIRO - RJ - BRASIL; 5. IMPG, UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Periodontitis is an oral chronic inflammatory disease, in response to the dysbiotic biofilm. It is capable of causing complete destruction of periodontal tissues. Both the oral dysbiosis and inflammatory process in periodontitis are associated with intestinal microbiome dysbiosis and inflammatory bowel diseases (IBD). Th17 cells play a role in the pathogenesis of IBD and are also related to the destruction of periodontal tissue. During inflammatory conditions, extracellular ATP activates P2X7 receptors, which culminates in the release of the pro-inflammatory cytokine IL-1 β . Our hypothesis is that the oral condition in periodontitis aggravates colitis via oral and hematogenous routes. Therefore, the P2X7 receptor could be directly involved in these axes, particularly in induction of the main cells involved, Th17 cells. Thus, the main goal is to investigate the role of the P2X7 receptor in oral-gut dysbiosis and the development of colitis in mice with ligature-induced periodontitis. An experimental animal model was used. A ligature was made around the first mandibular molar of Wild-type (WT) and P2X7 receptor-deficient C57BL/6 mice. After 14 days, colitis was induced by 1,5% sodium dextran sulfate (DSS) for 5 days, and they were euthanized 2 days later for a total of 21 days of ligature. They were divided in 4 groups each of both mice genotype: sham group, ligature group, DSS group and ligature+DSS group. Induced periodontitis led to alveolar bone loss to similar degrees between WT and P2X7^{-/-} mice. Nevertheless, regulatory T (Treg) cells frequency in the submandibular lymph node from WT ligature mice was decreased compared to WT sham mice, but not in P2X7^{-/-} groups. However, Treg cells in the mesenteric lymph node from WT ligature mice were increased, while in P2X7^{-/-} sham mice it was increased compared to WT mice. The initial results suggest that only the condition of induced periodontitis effectively modulated Treg cells in gut mice, independent of induced colitis. **Keywords:** Periodontitis; Inflammatory Bowel Disease; P2X7 receptor.

3328 - Category: CELLULAR IMMUNOLOGY (CE)

New insights of IL-33: This alarmin elicits LTC₄ synthesis in allergic inflammation via ST2-mediated activation of eosinophils

GARCIA, V.F.D.R.¹; FERREIRA, E.G.¹; DE SOUZA, Y.B.L.²; OLIVEIRA, J.F.²; DA SILVA, J.S.¹; TASMO, N.R.A.¹; GONÇALVES, L.B.¹; MUNIZ, V.D.S.¹; DE MELO, C.B.³; WERNECK, M.B.D.F.¹; DIAZ, B.L.¹; CANETTI, C.D.A.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE UNIGRANRIO AFYA, RIO DE JANEIRO - RJ - BRASIL; 3. IBMR, RIO DE JANEIRO - RJ - BRASIL.

Like pieces of a puzzle, IL-33, eosinophils and cysteinyl leukotrienes seem to come together and orchestrate allergic inflammation. While IL-33/ST2 receptor axis is known to activate some classical eosinophil functions, its ability to specifically trigger LTC₄ synthesis remains elusive. Here, employing a murine model of allergic inflammation, ST2 activation emerged as key step to LTC₄ synthesis, primarily achieved by lipid bodies-enriched eosinophils. Concurring, exogenous IL-33 elicited LTC₄ synthesis from activated eosinophils, both *in vivo* and from human cells *in vitro*. Thus, relevant to eosinophil-regulated environments, such IL-33/ST2-driven cellular effect may bear therapeutic potential as a target in cysteinyl leukotrienes-mediated conditions, showing another perspective and activity that this alarmin is capable of performing. Keywords: IL-33;Eosinophil;lipid body synthesizing LTC₄.

3334 Category: CELLULAR IMMUNOLOGY (CE)

LYMPHOID-DRIVEN METABOLIC SIGNALS ORCHESTRATE LPS-INDUCED STEATOSIS IN THE LIVER

OLIVEIRA, H.; ANTUNES, M.M.; PEDROSA, M.L.M.P.; COELHO, J.; RAMOS, G.P.; JÚNIOR, W.F.D.S.; CORRADI, P.S.; DE PAULA, C.; MENEZES, G.. UFMG, BELO HORIZONTE - MG - BRASIL.

INTRODUCTION: The liver is both a metabolic powerhouse and a key immunological organ. Despite their physical proximity, the functional relevance of having immune and metabolic systems co-localized remains largely unclear. In particular, how immune activation might reshape hepatocyte metabolic programs is not well understood. Toll-like receptor 4 (TLR4), present in both hepatocytes and immune cells, provides a unique model to investigate how immune-derived signals can influence liver metabolism. Here, we explore how systemic LPS challenge affects hepatic lipid homeostasis through immune mechanisms. **OBJECTIVE:** To dissect how TLR4-driven immune activation impacts hepatocyte metabolism, focusing on identifying immune compartments responsible for LPS-induced steatosis. **METHODS & RESULTS:** Male C57BL/6 mice received LPS and were analyzed after 24h. Intravital microscopy with BODIPY-labeled lipids and hepatocyte gene expression profiling revealed strong inflammation, hepatic lipid accumulation, and upregulated lipogenic pathways. Using conditional TLR4 knockouts - Albcre/TLR4flox (hepatocyte-specific) and LysMcre/TLR4flox (myeloid-specific) - we observed steatosis similar to wild-type mice, suggesting these cell types are not solely responsible. Targeted immune cell depletion refined this finding: neither Kupffer cell nor neutrophil depletion altered steatosis. In contrast, NSG-IL2null mice, with major reduced NK cells, showed striking attenuation of lipid accumulation. This points to lymphoid-derived signals, potentially from NK cells, as key mediators of LPS-induced steatosis. **CONCLUSION:** LPS-induced hepatic steatosis is not driven by hepatocyte- or myeloid-intrinsic TLR4 signaling, but through lymphoid-dependent mechanisms. These findings reveal an immunometabolic axis in the liver and highlight the role of lymphoid - particularly NK - cells in regulating hepatic lipid metabolism. Keywords: Liver immunometabolism;TLR4 signaling;Lymphoid-driven steatosis.

3349 - Category: CELLULAR IMMUNOLOGY (CE)

Cell therapy using extracellular vesicles from mesenchymal stromal cells against experimental infection of *Leishmania amazonensis* is dependent of IL10

FERNANDEZ, C.N.¹; DE ALMEIDA, D.B.²; GUEDES, H.L.D.M.². 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis is a global public health problem caused by pathogens of the *Leishmania* genus, which cause multiple types of clinical manifestations of the cutaneous form of leishmaniasis, but effective prevention and treatment are still lacking.

Previous data have demonstrated that therapy with extracellular vesicles of adipose tissue-derived mesenchymal stromal cells (AD-MSC-EVs) is a viable alternative for the treatment of cutaneous leishmaniasis, being effective in reducing lesions in C57BL/6 mice infected with *L. amazonensis*.

To understand whether the observed efficacy effects could be associated with cytokine regulation in the microenvironment, we sought to study the use of AD-MSC-EVs in C57BL/6 mice deficient in pro- and anti-inflammatory cytokines infected with *L. amazonensis* as a possible therapeutic model for the disease.

To perform the experiment, mice of the C57BL/6 wild-type (WT), C57BL/6 with interferon gamma knockout (IFN- γ KO) and IL-10 (IL-10 KO) strains were infected intradermally with 10 μ L of PBS containing 2×10^6 promastigotes of *L. amazonensis* and treated with three doses of 1×10^9 of AD-MSC-EVs intralesionally with one week gap, using as a control group animals treated with PBS.

As a result, it was observed that WT and IFN- γ KO treated with EVs presented reduction of the lesion thickness, however, IL-10 KO mice did not. When analyzing the images of the appearance of the lesions, it was observed that, in the groups treated with EVs, the evolution of the pathology occurred later in comparison to PBS, however IL-10 KO mice necrosis occurs in both groups treated with EVs and PBS at same time, while the animals treated only with PBS presented the development of the lesion earlier.

Therefore, we conclude that AD-MSC-EVs are effective in wound healing and are associated with delayed disease progression. We observed that this process does not occur in IL-10 KO mice, suggesting that EVs depend on this cytokine for its effectiveness. Keywords: Leishmaniasis; Extracellular vesicles; Mesenchymal stromal cells.

3352 - Category: CELLULAR IMMUNOLOGY (CE)

INVOLVEMENT OF THE mTOR PATHWAY IN EXPERIMENTAL IN VITRO INFECTION BY *Trypanosoma cruzi* IN MACROPHAGES

DE SOUZA, G.B.; SILVA, R.V.D.S.; RAMPINELLI, P.G.; MATEUS, L.C.O.; DE ALMEIDA, P.E.; CASTRO, J.M.D.A.; BIZARRO, H.D.D.S.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Chagas disease is an infection caused by the parasite *Trypanosoma cruzi* (*T. cruzi*) which affects between 6 and 7 million people worldwide. It is estimated that there are currently at least one million people infected by *T. cruzi* in Brazil. Macrophages are one of the first cells in the immune system to combat the parasite. Infected macrophages exhibit extensive formation of lipid bodies induced by specific signaling pathways involving recognition receptors, such as Toll-like receptors and others, and these organelles serve as sites of production of lipid mediators, modulating the host response. Previous studies showed that the mTOR pathway is activated in host cardiomyocytes after intracellular parasitic infection, including *T. cruzi*. Here, we evaluated the involvement of the mTOR pathway in modulating the macrophage response through the analysis of the biogenesis of lipid bodies, cytokine secretion, parasite replication, and production of reactive species of oxygen. For this purpose, THP-1 monocytes were cultured, differentiated into macrophages, infected with *T. cruzi* for 24 h, and treated with rapamycin, an inhibitor of mTORC1 (30 min before or 24 h after the infection). Our results demonstrated that *T. cruzi* infection was able to induce lipid body biogenesis, whose content is used by *T. cruzi* to enhance its replication, as well as increased production of TNF- α , interleukin-10 (IL-10), and hydrogen peroxide (H_2O_2), a microbicidal substance essential in combating *T. cruzi*. Interestingly, treatment with rapamycin was able to reduce cytokine secretion of IL-10, as well as H_2O_2 production and parasite replication, by interfering in the biogenesis of lipid bodies. Thus, inhibition of the mTOR pathway may be a potential target for therapy against Chagas disease. Keywords: *Trypanosoma cruzi*; Lipid bodies; mTOR.

3360 - Category: CELLULAR IMMUNOLOGY (CE)

Alterations in adaptive immune responses in patients with Wiskott-Aldrich syndrome: impairment of effector T cells and regulatory B cells

DE LIMA, L.G.R.¹; GUANAES, J.O.F.²; DE SIQUEIRA, L.H.²; GESU, R.S.W.D.³; YAMAGUTI-HAYAKAWA, G.G.²; VILELA, M.M.D.S.¹; OZELO, M.C.¹. 1. UNIVERSITY OF CAMPINAS, UNICAMP, CAMPINAS - SP - BRASIL; 2. HEMOCENTRO UNICAMP, UNIVERSITY OF CAMPINAS,, CAMPINAS - SP - BRASIL; 3. CONCEIÇÃO CHILDREN'S HOSPITAL (HCC), PORTO ALEGRE - RS - BRASIL.

Wiskott-Aldrich syndrome (WAS) is a rare immunodeficiency with different severity levels, classified by scores that reflect the severity of symptoms. The mildest form is X-linked thrombocytopenia (XLT), and more severe cases range from scores 3 to 5 (WAS). We aimed to compare cellular immune responses of patients with XLT and WAS. We included 5 patients diagnosed with XLT or WAS who are longitudinally followed. We evaluated clinical data and the peripheral blood mononuclear cells (PBMCs) response. The PBMCs were seeded into 96-well plates containing RPMI-1640 medium and cultured for 24 hours under stimulation with LPS, IL1 β , and IL2. Intracellular trafficking was blocked with GolgiPlug/GolgiStop 6 hours before the assay, and the cells were then stained with labeled antibodies to evaluate the immune response. We evaluated CD4⁺ and CD8⁺ responses assessed by cytokine frequency (IL2, IL4, IL6, IL10, IL17A, IL21, TNF α , FoxP3 and perforin), and Bregs based on IL10 production in CD19⁺CD24⁺CD38⁺. Two XLT patients (score 2) exhibited recurrent respiratory infections. One WAS patient (score 5) presented severe infections and autoimmunity, while another (score 3) showed only eczema. When LPS-stimulated, PBMCs from XLT/WAS patients showed decreased frequency of CD8⁺ T cells (p = 0.01). When compared with control, XLT/WAS group presented reduction in memory B cells (CD19⁺CD27⁺CD38⁺, p = 0.03), following lower frequency of CD71⁺ (p = 0.04) and IL10 from total CD19⁺ (p = 0.02). XLT/WAS patients presented higher frequency of IL2 (p = 0.02) compared to controls at unstimulated group. Also, there was lower IL10 frequency on CD19⁺CD24⁺CD38⁺ from XLT/WAS patients, which is even lower in WAS group (1.8-fold decrease). The WAS group (with more severe phenotype) has higher IL21 (13-fold increase) and IL10 (3-fold increase) frequency in T CD4⁺ when compared to XLT patients or controls. Our data suggest that WAS patients have impaired Breg response than XLT, accompanied by IL21 increase. Keywords: Wiskott-Aldrich syndrome;Regulatory B cells;X-linked thrombocytopenia.

3383 - Category: CELLULAR IMMUNOLOGY (CE)

Anti-inflammatory evaluation of Croton lechleri latex originating from the Brazilian Amazon

TEZZA, L.C.¹; DE OLIVEIRA, R.C.²; RAMOS, E.D.S.²; NETO, J.A.C.³; DE SALES, B.L.⁴; CATÃO, C.L.D.S.²; SOUSA, G.C.C.⁵; BASTOS, I.D.S.⁶; DA COSTA, A.G.²; LOPES, A.L.R.B.². 1. LABORATORIO REUNIDOS DA AMAZÔNIA, MANAUS - AM - BRASIL; 2. UNIVERSIDADE FEDERAL DO AMAZONAS - UFAM, MANAUS - AM - BRASIL; 3. UNIVERSIDADE ESTADUAL DO AMAZONAS - UEA, MANAUS - AM - BRASIL; 4. DIRETORIA DE ENSINO E PESQUISA, FUNDAÇÃO HOSPITALAR DE HEMATOLOGIA E HEMOTERAPIA DO AMAZONAS (HEMOAM), MANAUS - AM - BRASIL; 5. LABORATÓRIOS REUNIDOS DA AMAZÔNIA, MANAUS - AM - BRASIL; 6. INSTITUTO LEÔNIDAS E MARIA DEANE – ILM, FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), MANAUS - AM - BRASIL.

Introduction: Croton lechleri, known as "dragon's blood," is used by Amazonian communities to treat internal scars, bone cancer, and tuberculosis. Since its traditional use is lacking scientific evidence, this study evaluated the effects of latex at different doses in macrophages derived from the THP-1 lineage, including cytotoxicity and modulation of pro- and anti-inflammatory cytokines. **Methods:** Croton lechleri latex was transesterified and characterized by gas chromatography-mass spectrometry. Cell viability was investigated by the MTT assay in latex-treated, macrophage-differentiated THP-1 cells. The cytokine profile was quantified using the BD Cytometric Bead Array Human Th1/Th2/Th17 kit. Macrophages were pretreated for 24 hours and then stimulated with lipopolysaccharide (1 mg/mL) for another 24 hours, and the supernatant was collected for analysis. **Results:** The results obtained with concentrations of 25, 50, 75, and 100 μ g/ml of Brazilian Croton lechleri latex revealed no significant increase in the cytokines IFN- γ , IL-4, IL-2, and IL-17, suggesting that these specific cytokines did not promote a robust immune response. However, a significant increase in the production of IL-6, TNF- α , and IL-10 was observed, particularly at concentrations of 50, 75, and 100 μ g/ml. The results were expressed in pg/mL of total protein. A probability value of less than 0.05 was used as the criterion for statistical significance (one-way ANOVA; Tukey's multiple comparison post-test). All concentrations were evaluated in triplicate for each individual concentration. **Conclusion:** Our results indicate that pretreatment with Brazilian C. lechleri latex results in a significant and concentration-dependent increase in IL-6 concentration in THP-1 cells differentiated into macrophages. This finding suggests the potential efficacy of the traditional use of the sap, possibly due to the immunomodulatory properties of IL-6. Keywords: cytokines;natural drugs;immunomodulatory properties.

3386 - Category: CELLULAR IMMUNOLOGY (CE)

The role of STING signaling in osteoblast and inflammatory bone resorption

DA SILVA, M.L.M.¹; RITEAU, N.²; ALVES, S.Y.F.³. 1. FORP-USP, RIBEIRÃO PRETO - SP - BRASIL; 2. NATIONAL CENTRE FOR SCIENTIFIC RESEARCH (CNRS), RIBEIRÃO PRETO - SP - BRASIL; 3. FCFRP-USP, RIBEIRÃO PRETO - SP - BRASIL.

Bone tissue integrity depends on a well-regulated balance between osteoblast and osteoclast activity, a process that can be disrupted by chronic inflammation. The cGAS-STING pathway is a key cytosolic DNA-sensing mechanism that bridges innate immunity and inflammation. This project aims to investigate the role of the cGAS-STING pathway in bone cells in vitro. Specifically, we aim to evaluate whether osteoblasts activated with STING agonists interfere with its differentiation and also with the interaction between osteoblast-osteoclast. Cell viability assay was performed using primary murine osteoblasts treated with STING agonists (c-di-AMP, c-di-GMP, and DMXAA) at concentrations of 1, 3, and 10 μ M for 2, 24, and 72 hours. The STING agonists exhibited distinct and time-dependent effects on osteoblast viability. Notably, while c-di-AMP reduced cell viability after 72 hours of treatment, c-di-GMP and DMXAA promoted it at 24 and 72 hours, respectively, suggesting complex regulatory roles beyond simple cytotoxicity. We confirmed the activation of cGAS-STING pathway by western blotting, on osteoblasts treated with 3 μ M of each agonist for 2 and 24 hours. We evaluated the expression of genes related to the STING pathway activation (IFN β and TMEM173), osteoblast function (RUNX2 and ALP), and osteoclast regulation (RANKL and OPG) by RT-qPCR. Our data confirm the activation of STING by c-di-GMP and DMXAA by the increased expression of IFN β . While DMXAA increased the expression of osteoblast marker genes such as ALP and RUNX2, c-di-GMP stimulated the expression of RANKL by osteoblasts, which is a crucial tool to control osteoclastogenesis. These preliminary findings suggest that the cGAS-STING pathway in osteoblasts may be a key modulator and capable of directly influencing the osteoimmune interactions regulating osteoclast differentiation and controlling bone homeostasis. Keywords: cGAS-STING pathway; osteoimmunology; osteoclastogenesis.

3410 - Category: CELLULAR IMMUNOLOGY (CE)

Analysis of senescent T cells in sickle cell disease patients.

HIROSE, G.¹; RÓS, F.A.²; MAIO, K.T.²; COUTO, S.C.F.³; ROCHA, V.G.⁴. 1. LABORATORY OF MEDICAL INVESTIGATION IN PATHOGENESIS AND DIRECTED THERAPY IN ONCO-IMMUNO-HEMATOLOGY (LIM-31), HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA USP; INSTITUTO DE CIÊNCIAS BIOMÉDICAS USP, SÃO PAULO, SP - SP - BRASIL; 2. LABORATORY OF MEDICAL INVESTIGATION IN PATHOGENESIS AND DIRECTED THERAPY IN ONCO-IMMUNO-HEMATOLOGY (LIM-31), HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA USP (HCFM-USP), SÃO PAULO - SP - BRASIL; 3. LABORATORY OF MEDICAL INVESTIGATION IN PATHOGENESIS AND DIRECTED THERAPY IN ONCO-IMMUNO-HEMATOLOGY (LIM-31), FUNDAÇÃO PRÓ-SANGUE-HEMOCENTRO DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. LABORATORY OF MEDICAL INVESTIGATION IN PATHOGENESIS AND DIRECTED THERAPY IN ONCO-IMMUNO-HEMATOLOGY (LIM-31), HCFM-USP; FUNDAÇÃO PRÓ-SANGUE-HEMOCENTRO DE SÃO PAULO; INSTITUTO D'OR DE ENSINO E PESQUISA, SÃO PAULO - SP - BRASIL.

Accumulation of senescent immune cells (SICs) is a natural process observed during aging. SICs are generally characterized as non-proliferative and exhibit phenotypic, metabolic and functional alterations. However, several studies have reported an early accumulation of SICs, particularly senescent T lymphocytes (Tsen), in patients with diseases associated with chronic inflammatory states, such as cancer, rheumatoid arthritis, and HIV infection. In this context, sickle cell disease (SCD) stands out due to its chronic pro-inflammatory nature, and multi-organ dysfunction. Despite some studies demonstrating telomere shortening in peripheral blood mononuclear cells (PBMCs) from SCD patients, the senescence profile of lymphocytes in these individuals remains poorly characterized. This study aimed to assess the presence of CD4⁺ and CD8⁺ Tsen cells among PBMCs from SCD patients (n=18) and healthy blood donors (HD) (n=14). Analyses were performed using immunophenotypic profiling based on surface markers associated with senescence, CD27, CD28, CD57, and KLRG1, as well as the accumulation of senescence-associated β -galactosidase (SA- β -Gal), both assessed by flow cytometry. Preliminary results show that SCD patients exhibit a lower percentage of CD3⁺ T cells (25.03% $\pm$ 9.780) compared to HDs (44.73% $\pm$ 7.901), along with a reduced proportion of CD4⁺ Tsen (1.312% $\pm$ 2.469) and CD8⁺ Tsen cells (11.39% $\pm$ 6.682) relative to HD (CD4⁺ 3.83% $\pm$ 5.42; CD8⁺ 16.91% $\pm$ 11.49). Interestingly, the proportion of CD4⁺ and CD8⁺ SA- β -Gal⁺ T cells was higher in SCD patients (21.70% $\pm$ 14.21 and 32.60% $\pm$ 18.53, respectively) than in HD (14.96% $\pm$ 5.938 and 24.99% $\pm$ 8.036, respectively). These findings suggest that SCD or its various treatment regimens may alter Tsen dynamics compared to HD. Nonetheless, increasing the sample size of both patient and control groups will be essential to allow stratification by age and treatment protocols, enabling a more comprehensive understanding of this biological phenomenon. Keywords: Immunosenescence; T-cell senescence; Sickle Cell Disease.

3419 - Category: CELLULAR IMMUNOLOGY (CE)

Initial comparison of B lymphocytes cells profile of elderly active individuals and sedentary pars

BARBOSA, B.L.¹; SOUZA, O.F.¹; DO AMARAL, J.B.¹; BACHI, A.L.L.²; POPI, A.F.¹. 1. UNIVERSIDADE FEDERAL DE SÃO PAULO - UNIFESP, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE SANTO AMARO - UNISA, SÃO PAULO - SP - BRASIL.

The immune system is influenced by many factors, including individual lifestyle. Physical exercise is certainly an important component of healthy aging, contributing to prevent or mitigate diseases and cognitive impairment. It is known that physical exercise also modulates immunological functions. T and B lymphocytes, as components of the adaptive immune system, are suitable for this modulation. Using flow cytometry, PBMC of individuals between ages of 65 and 80 years who exercise regularly and those who do not (control group) were submitted to evaluation of B and T lymphocytes populations. It was not observed any statistical significant differences in the quantity of cells in CD3+ population between control (64,1%±0) and exercise group (27,21%±31,71), neither in CD19+ population (32,9%±0 vs. 8,8%±5,1). Gates for B2 cells CD19+CD23+ (4,83±0 vs. 20,1±18,9) and B1 cells CD19+CD23- (95,6±0 vs. 79,3±18,5) showed no clear difference between both groups. It was not observed a clear correlation between cell numbers and aging, in the group exercise (R2=0,02 for CD3+ cells and R2=0,07 for CD19+ cells). This study has limitations, since it used a small number of controls. We pretend to include more sedentary elderly individuals who are being recruited to increase the number of observations. Thus, more studies must be conducted to indicate if chronic physical exercise modulates immune cell numbers in the elderly population. Keywords: lymphocytes; exercise; immunosenescence.

3421 - Category: CELLULAR IMMUNOLOGY (CE)

Disruption of the guanine exchange factor RasGEF1b impairs viral spread and cytoskeletal remodeling during MHV-3 infection in macrophages

SILVA, V.R.D.P.E.¹; DOS SANTOS, C.A.C.²; JÚNIOR, C.M.Q.³; DE OLIVEIRA, I.M.⁴; CLEMENTE, B.T.⁵; ROCHA, F.E.O.³; COSTA, V.V.³; DA SILVA, A.M.³. 1. PUC MINAS, BELO HORIZONTE - MG - BRASIL; 2. UFMG, GUAJARÁ-MIRIM - RO - BRASIL; 3. UFMG, BELO HORIZONTE - MG - BRASIL; 4. UFMG, USP, RIBEIRÃO PRETO - SP - BRASIL; 5. PUC MINAS, UFMG, BELO HORIZONTE - MG - BRASIL.

The reorganization of the actin cytoskeleton induced by betacoronavirus in macrophages is crucial for efficient viral replication and spread. In this study, we identified a key role for the Ras guanine exchange factor member 1b (RasGEF1b) in mediating murine hepatitis virus 3 (MHV-3) infection and replication in vitro. Using bone marrow-derived macrophages (BMDMs) lacking RasGEF1b as well as shRNA-mediated knockdown (KD) of RasGEF1b in RAW264.7 cells, we observed a marked reduction in cell size and retracted cell morphology compared to controls. Confocal microscopy studies revealed that RasGEF1b silencing disrupted actin cytoskeleton organization and altered cellular morphology. Following MHV-3 infection, RasGEF1b KD cells exhibited significantly reduced viral titers across different multiplicity of infection (MOI's), and showed increased viability at 24 hours post-infection. Moreover, these cells presented impaired formation of membrane protrusions and syncytia, structures commonly associated with viral dissemination and immune evasion. RasGEF1b-KO animals exhibited a reduced neutrophil-to-lymphocyte ratio (NLR), markedly lower pulmonary viral titers, and attenuated tissue damage, as shown by histopathological analysis. Bronchoalveolar lavage fluid (BALF) examination revealed decreased leukocyte infiltration and a lower neutrophil proportion in KO mice compared to wild type (WT). Despite milder lung injury, KO mice displayed elevated inflammatory cytokine levels along with increased anti-inflammatory IL-10, indicating that RasGEF1b deficiency modulates the inflammatory response in a manner that favors tissue protection. Collectively, our findings suggest that RasGEF1b is a critical regulator of macrophage susceptibility to MHV-3 infection, potentially by modulating actin cytoskeleton dynamics essential for viral replication and spread. Keywords: MHV-3; Macrophage; Actin cytoskeleton.

3424 - Category: CELLULAR IMMUNOLOGY (CE)

Cytotoxic effect of T cells carrying human or humanized CAR molecules targeting CD20 against Burkitt's lymphoma model in vitro

MARQUES, J.D.D.P.C.¹; BONAMINO, M.H.¹; MARANHÃO, A.Q.²; STUDART, I.C.²; SOUSA, I.G.². 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

CAR-T cell therapy is based on the genetic modification of T lymphocytes for the expression of transgenic receptors, enabling the cells to recognize a specific antigen and exert a cytotoxic response. Many CAR-T products are already approved for B-cell neoplasia treatment, mainly targeting CD19 and BCMA. Traditional CAR molecules are composed of a signaling intracellular domain and an extracellular domain responsible for interacting with the target. This extracellular domain is commonly a scFv derived from an IgG. Although Rituximab was the first monoclonal antibody approved for cancer treatment by the FDA, targeting CD20, no CAR-T product derived from this antibody was approved. The objectives of this work were to express CAR molecules containing scfv from Rituximab, humanized versions of it and new human scFvs and evaluate the cytotoxic effect of the respective CAR-T cells in co-culture with Raji cells, which presents CD20 in its membrane. The human sequences were obtained through a phage display using a human library. The humanized versions were obtained from the Rituximab sequence by transplanting its CDRs into different human germline VH and VL genes. The CAR gene was cloned into the pT4 expression vector, a plasmid carrying Sleeping Beauty transposon. PBMC were obtained through gradient density separation from healthy blood donors, and the cells were electroporated with a mix containing plasmids for the transposase and the transposon carrying the CARs genes. The T-cells were expanded and were co-cultivated with Raji cells expressing GFP. The cytotoxic effect was measured by counting the GFP+ events present in the co-culture over 4 days through flow cytometry. Rituximab derived CARs were successfully expressed in T cells, including humanized versions with a range of 5% to 25% expressions measured with flow cytometry. Human sequences were expressed as well with a maximum of 8% expression. Data from cytotoxic assays are still being generated. Keywords: CAR-T;CD20;ALL-B.

3425 - Category: CELLULAR IMMUNOLOGY (CE)

ANALYSIS OF THE IMPACT OF HYPERGLYCEMIA ON THE BIOLOGY OF T LYMPHOCYTES IN INDIVIDUALS INFECTED WITH HTLV-1

GONCALVES, M.D.S.¹; DA SILVA, M.T.T.²; LIMA, M.A.²; LEITE, A.C.C.B.²; DE LIMA, J.E.N.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. INI/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: The Human T-cell lymphotropic virus type 1 (HTLV-1) is a complex retrovirus that causes a chronic, slowly progressive infection. Hyperglycemia gradually increases with age, and this glycemic imbalance affects the immune response. In addition to the onset of related diseases being favored with age, viral reactivation is strongly influenced by glucose, as suppression of viral transcription was detected in CD4+ T cells in low glucose medium. There is also evidence of a correlation between high HTLV proviral load and type 2 diabetes risk. High glucose concentrations in vitro impair the antiviral response of CD8+ T lymphocytes and decrease the activation of CD4+ T cells. However, the mechanisms triggered by hyperglycemia during HTLV-1 infection and its effects on cellular dynamics remain unknown. Thus, this study aims to investigate the influence of hyperglycemia on the phenotype and cytokine production of T cell subpopulations in patients infected with HTLV-1. **Methods and Results:** PBMCs from HTLV patients (HTLV+) and uninfected individuals (NI) donors were incubated for 48 hours in different glucose concentrations (norm glucose: 5 mM, intermediate glucose: 15 mM, high glucose: 30 mM). Then, the immunophenotyping of T cells was performed by flow cytometry. The results obtained did not show a significant change in the frequency of cellular subpopulations (CD4 and CD8: Memory, Effector, Naïve, and Exhausted) in the NI and HTLV+ experimental groups after glucose treatment. The basal Granzyme B, IFN- γ , and TNF- α production also remained unchanged in the presence of high glucose. Using an HTLV-1-infected cell line (MT2), we did not detect proliferation enhancement; however, glucose increased cell metabolism, and radical oxygen species were measured using dihidrorodamine 123 probe. **Conclusion:** Together, our results suggest that hyperglycemia may not affect the T cell subpopulations of HTLV-1 patients but can alter cell metabolism and redox state. Keywords: HTLV-1;Hyperglycemia ;Metabolism.

3456 - Category: CELLULAR IMMUNOLOGY (CE)

Platelet-monocyte and platelet-neutrophil interactions associate with thromboinflammation and cognitive impairment in long COVID

DE PAULA, M.M.L.¹; OLIVEIRA, R.T.R.¹; DOS SANTOS, J.P.R.S.¹; GOMES, M.T.¹; DIB, P.R.B.¹; SHIGAEFF, N.¹; TOSCANO, E.C.D.B.¹; BOZZA, P.T.²; WEYRICH, A.³; HOTTZ, E.¹. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 3. OKLAHOMA MEDICAL RESEARCH FOUNDATION, OKLAHOMA - ESTADOS UNIDOS DA AMERICA.

Long COVID is characterized by persistence of symptoms years after the resolution of the acute infection by the SARS-CoV-2, associating with cognitive impairment and coagulatory disorders. The relationship between thromboinflammation and cognitive impairment in long COVID remains elusive. We prospectively verified associations between myeloid cell response, platelet aggregation, and cognitive impairment in patients characterized with long COVID. Patients were evaluated in different time points, denominated as T1 at 281 (± 60.28), T2 at 370 (± 83.76) and T3 at 488 (± 71.08) days post infection. We utilized samples of pre-pandemic patients and COVID-19 recovered subjects which did not developed long COVID as controls. Through flow cytometry, we verified monocyte and neutrophil Tissue Factor (TF) and CD11b expression and aggregation with platelets. Results show higher TF expression and aggregation with platelet in monocytes and neutrophils. Platelet interacting cells (CD41+) express more TF compared to CD41- cells, and monocytes in this context also expressed higher CD11b. Monocyte adhesion assay on fibrinogen and P-selectin suggests monocytes of long COVID patients show no difference compared to recovered patients. Platelet-monocyte interactions and TF expression reduce in the T3 time point compared to T1, suggesting inflammation resolution. Classical monocytes were negatively correlated with work memory (Digit span R test, $\beta = -0.090$, $p = 0.045$) and CD41+ non-classical monocytes (CD14^{low} CD16⁺) with memory impairment (HVLt trial R test, $\beta = -0.017$, $p = 0.044$). Neutrophil TF expression negatively correlated with memory (HVLt trial R test, $\beta = -0.031$, $p = 0.031$) and attention (Stroop 3 test, $\beta = -0.086$, $p = 0.001$), and CD41+ neutrophils with work memory (Symbol digit test, $\beta = -0.031$, $p = 0.019$) and attention (Stroop 3 test, $\beta = -0.551$, $p = 0.008$). These data contributes to the comprehension of platelet-myeloid cell communication in coagulatory disorders associated with cognitive impairment. Keywords: Platelets; Long COVID; Cognitive Impairment.

3459 - Category: CELLULAR IMMUNOLOGY (CE)

Key Genes and Pathways Modulated by Hyperglycemia during MRSA Skin Infection

LIMA, L.K.S.C.¹; PENTEADO, L.D.A.¹; SEREZANI, C.H.²; OLIVEIRA, K.C.¹; RAIMUNDO, B.V.B.¹; DE MEDEIROS, A.I.¹. 1. UNESP, ARARAQUARA - SP - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL.

High glucose levels are associated with impairment of the immune cell activation, such as neutrophil, macrophage and natural killer cell functions, thus, creating a proper environment for bacterial proliferation. Consequently, diabetic patients are more susceptible to methicillin-resistant *Staphylococcus aureus* (MRSA) skin infections, posing significant therapeutic challenges. This study aimed to identify key genes and biological pathways modulated by hyperglycemia during MRSA skin infection through RNA sequencing (RNAseq) and bioinformatics. Total mRNA was isolated using the Direct-zol RNA Microprep kit, quantified, and amplified with the Ovation RNA-Seq System V2 kit. The resulting RNA was then sequenced using the Illumina NovaSeq 6000. Differential gene expression analysis was performed in R. Gene Set Enrichment Analysis (GSEA) was used to identify significantly enriched biological processes in the Gene Ontology (GO). A network integrating the main pathways and their top differentially expressed genes was constructed using ClusterProfiler. Our analysis revealed that the three most downregulated pathways were inflammatory response, defense response, and myeloid leukocyte activation, indicating suppression of key immune functions under hyperglycemic conditions. The top-upregulated enriched pathways were those related to epidermis development, osteoblast differentiation, and tissue development, suggesting compensatory tissue remodeling and repair mechanisms. Notably, the most downregulated genes included *Ptgis*, *Nlrp12*, *Cxcr2*, *Chil1*, *Acod1*, *Tlr7*, *Fpr2*, *Ddx60*, *Ifnlr1*, *Mmp12*, *Clec5a*, *Lyst*, *Cd177*, and *Bnip3*; while the most upregulated genes were *Apela*, *Krt35*, *Krt87*, *Edn3*, *Rspo2*, *Rspo3*, and *Nell1*. These findings highlight the impact of hyperglycemia on host defense and tissue remodeling during MRSA skin infection. Our next steps will involve analyzing protein-protein interaction networks, as well as *in vitro* validation of the top differentially expressed genes. Keywords: RNA sequencing; Diabetes; Methicillin-resistant *Staphylococcus aureus* (MRSA).

3460 - Category: CELLULAR IMMUNOLOGY (CE)

Ovalbumin-expressing Neuroblastoma Cell line: a tool to Detect Autoreactive CD4⁺ T Cells During Efferocytosis

COSTA, A.L.M.D.S.; RAIMUNDO, B.V.B.; OLIVEIRA, K.C.; DE SOUZA, T.M.; DE MEDEIROS, A.I.. UNESP, ARARAQUARA - SP - BRASIL.

Efferocytosis of infected apoptotic cells (iAC) by dendritic cells (DCs) enhances bacterial clearance but can also activate autoreactive T cells. Zika virus (ZIKV) infection induces massive neuronal apoptosis, causing neuropathogenesis such as microcephaly and Guillain-Barré syndrome. The degradation of infected apoptotic cells during efferocytosis may be an important source of self-antigens that potentially trigger autoimmune diseases. In this study, we aimed to generate a stable transgenic neuroblastoma cell line expressing ovalbumin (OVA), to identify autoreactive CD4⁺ T cells activated during efferocytosis. Neuroblastoma cell line (SH-SY5Y) was engineered to express OVA as a model of self-antigen through lentiviral transduction. The plasmids containing genes that encode the OVA-coding (pLenti-OVA-eGFP), a viral envelope protein (pMD2.G), and others related to essential viral proteins (psPAX2) were cloned and then amplified in *E. coli* DH5 α . The plasmids were transfected into HEK 293T cells to pack viral particles, and the neuroblastoma cells were incubated with the supernatant for transduction. The percentage of cells expressing OVA-GFP⁺ was evaluated by flow cytometry and microscopy. Preliminary results demonstrated low transduction efficiency (~10.5% GFP⁺), likely due to the low plasmid concentration. Next, we conducted another plasmid recovery protocol, resulting in a higher final concentration and potentially improved plasmid quality [~400 ng/ μ L]. Then, new rounds of transfection and transduction have been evaluated to validate the improvements in OVA-GFP⁺ expression. Next, we will sort GFP-transduced live cells for subsequent culture and then submit them to radiation to induce apoptosis and efferocytosis assay. Together, generating stable OVA-expressing transgenic cell lines allows us to assess the impact of efferocytosis on activating autoreactive CD4⁺ OT-II cells and potentially breaking immune tolerance in the context of infection. Keywords: CD4⁺ autoreactive T cells; Autoimmunity; Efferocytosis.

3469 - Category: CELLULAR IMMUNOLOGY (CE)

Regulation of Lipid Droplets and Inflammatory Lipid Mediators by Bioactive Flavonoid Compounds

FIRMINO, L.D.S.L.; WANDERLEY, J.L.. UENF, CAMPOS DOS GOYTACAZES - RJ - BRASIL.

Lipid droplets (LDs) are multifunctional organelles that play central roles in cellular signaling, lipid metabolism, and the regulation of inflammatory mediator production, including eicosanoids. They serve as platforms for enzymes, growth factors, GTPases, and kinases, making them strategic for managing inflammatory and infectious processes. Bioflavonoids, such as rutin and quercetin, are phytochemicals with antioxidant, anti-inflammatory, antiallergic, and antitumor properties.

In this study, we investigated whether rutin and quercetin regulate prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) production via LD modulation in lipopolysaccharide (LPS)-activated macrophages over 6 hours. LDs were quantified by optical microscopy and flow cytometry, and eicosanoids were measured using competitive ELISA.

Our results reveal distinct mechanisms for each compound: quercetin reduced LD number by around 23% in cells activated with 10 μ g/mL LPS, but not under high-intensity inflammation (100 μ g/mL LPS), suggesting greater efficacy under moderate inflammatory conditions. Rutin did not significantly affect LD number under high inflammation, but significantly decreased LTB4 by approximately 89% and PGE2 by around 50% in cells treated with 100 μ g/mL LPS, indicating a different regulatory pathway.

We conclude that the anti-inflammatory activity of these bioflavonoids is linked to LD modulation and eicosanoid synthesis. Quercetin primarily modulates LD biogenesis, whereas rutin exerts a stronger effect on eicosanoid production, highlighting the potential of these compounds as candidates for anti-inflammatory therapeutic strategies. Keywords: lipid bodies; eicosanoids; bioflavonoids.

3500 - Category: CELLULAR IMMUNOLOGY (CE)

Characterization of molecular and immune cell modifications in skin and adipose tissue that occur with aging in C57BL/6 mice and the possible influence on delayed skin tissue repair

SILVA, J.K.A.D.O.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Since the last century, there has been a significant increase in life expectancy. Consequently, population aging is often accompanied by some comorbidity, primarily due to organic tissue fragility and replication damage. Among the organs most affected by these changes is the skin, which is exposed to recurring wounds.

Currently, approximately 2% of the world's population lives with a skin wound, the majority of whom are elderly.

Despite the significant impact on quality of life and the healthcare costs this condition entails, there is still no gold standard treatment that aids in the temporal reestablishment of skin healing.

Therefore, there is an urgent need to understand the physiology of aging to develop new therapeutic approaches for adequate healing.

Thus, our group's hypothesis is that cellular senescence and changes in the proportions of cell types in the skin and adipose tissue directly impact the delayed inflammatory phase of healing. To this end, we collected intact skin from young and aged mice and, using qPCR, observed an increase in mRNA encoding proteins p16, p21, mmp9, and cxcl1. Regarding the cellular repertoire of intact skin, cytometry revealed a decrease in Langerhans cells. We also observed a decrease in the number of viable adipose stem cells (ASC) in aged animals. Given these changes in aged intact skin, we performed a surgical skin wound experiment and observed that, starting on the third day after surgery, the aged animals exhibited delayed wound closure. To study the possible cellular differences found in the scar of aged animals, we performed cytometry on the wound on the seventh day after surgery and observed a decrease in the neutrophil and macrophage, and an increase in CD4⁺ T lymphocytes and T $\gamma\delta$ V γ 5. We believe that this delay in healing time in the elderly is mainly due to the basal decrease in Langerhans and T $\gamma\delta$ cells already in the inflammatory phase, in addition to the failure of paracrine communication between ASCs in the skin-adipose tissue axis.

Keywords: Senescence; Skin wound healing; Innate immunity.

3506 - Category: CELLULAR IMMUNOLOGY (CE)

STING activation triggers endoplasmic reticulum stress and promotes renal fibrosis

SILVA, M.A.¹; DHILLON, P.²; NAVARRO, A.S.²; MUKHI, D.²; KOLLIGUNDLA, L.P.²; ABEDINI, A.²; LEVINSHON, J.²; DUMOULIN, B.²; MINER, J.J.²; CAMARA, N.O.¹; SUSZTAK, K.². 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA - ESTADOS UNIDOS DA AMERICA.

Background: Endoplasmic reticulum (ER) stress is crucial in the pathogenesis of kidney disease, yet the mechanisms initiating ER stress remain largely unexplored. Viruses are known to manipulate ER-related processes, potentially aiding their replication and pathogenesis. The adaptor molecule STING, which detects double-strand DNA in viral and bacterial infections, has also been implicated in sterile chronic kidney inflammation and may influence ER stress. Given these insights, we investigated the relationship between STING, ER stress, and the development of kidney disease.

Methods: We utilized STING N153S mice, which exhibit constitutive STING activation, and mice with conditional STING deletion in kidney tubules. In vitro experiments included silencing of STING or ER stress pathways, and molecular events were analyzed through immunofluorescence, qPCR, Western blotting, and immunoprecipitation techniques. Gene expression data were scrutinized through RNA sequencing from human control and diseased samples, as well as mouse kidney disease models.

Results: In both cisplatin and UUO models, we observed consistent activation of STING and the ER stress-activated eIF2 α kinase (PERK) pathway. STING was found to physically interact with and activate PERK in tubule cell, leading to ER stress and subsequent renal fibroinflammation. Conversely, deletion of STING or inhibition of PERK provided protection against disease development. sc-RNA-Seq and spatial transcriptomic analysis of human CKD patients and mouse kidney injury models reinforced the association between renal dysfunction and activation of the ER stress pathway.

Conclusion: Our findings reveal a novel mechanism whereby STING activation triggers ER stress via the PERK pathway, resulting in kidney tubule injury and fibrosis. This breakthrough offers promising avenues for developing strategies to modulate ER stress and create effective therapies for fibroinflammatory kidney diseases.

Keywords: Kidney fibrosis; nucleic acid sensors; renal epithelial cell.

3507 - Category: CELLULAR IMMUNOLOGY (CE)

Potential of Extracellular Vesicles from Adipose Mesenchymal Stromal cells as a novel alternative therapy for cutaneous leishmaniasis from leishmania amazonensis

DE ALMEIDA, D.B.¹; FERNANDEZ, C.N.²; RAMOS, T.D.¹; GUEDES, H.L.D.M.¹. 1. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Background: Leishmaniasis is a neglected disease caused by protozoa of the genus *Leishmania*. One of its characteristics is the host's immune response imbalance to favor the parasite's survival. In this context, the use of adipose mesenchymal stromal cells (AD-MSCs) and some of their biological products such as their Extracellular Vesicles (AD-MSC-EVs) may be a viable therapeutic strategy, given their already described immunomodulatory potential. Aims: this work aims to investigate whether AD-MSC-EVs have the property of controlling the experimental infection induced by *Leishmania amazonensis* similar to full cell treatment and to investigate therapeutical mechanisms. Methods: Adipose-tissue derived Stromal Cells (AD-MSC-EVs) from C57BL/6 mice were collected and cultured until 4th passage and its extracellular vesicles (AD-MSC-EVs) were harvested, characterized and used in experimental *L. amazonensis* infection both in vitro and in vivo. Effects were evaluated by FACs, ELISA and Limited Dillution Assay (LDA). CEUA: 110/17 Results: Treatment with AD-MSC-EVs in vitro successfully managed to reduce the number of amastigotes per macrophage and the percentage of infected Macrophages in 24h, 48h and 72h of interaction in the early times of infection load through the generation of reactive oxygen species (ROS), independent of nitric oxide (NO). AD-MSC-EVs treatment in vivo partially controls the lesion size development, without affecting parasite load, while also decreasing levels of IgG, IgM, and cytotoxic CD8+ T cells. Additionally, combined therapy with pentavalent antimonial further reduced lesion size but did not impact the parasite load. Conclusion: Taken together, our data suggests Extracellular Vesicles from AD-MSC-EVs as a potential alternative platform for treatment of cutaneous leishmaniasis induced by *L. amazonensis*. However, further studies are required to understand fully the Mechanisms involved in AD-MSC-Evs efficacy. Keywords: Alternative Therapy; Extracellular Vesicles; cutaneous leishmaniasis.

2932 - Category: CHEMOKINES AND TRAFFICKING (CT)

Acute Resistance Exercise Elicits Similar Cytokine-Mediated Responses Across Omnivorous and Vegetarian Diets, Despite No Change in Innate Immune Function

SALVALÁGIO, B.R.¹; VOLPE-FIX, A.R.¹; GHAFOURI, B.²; SILVA, B.M.¹; MARGINEAN, D.²; THOMATIELI-SANTOS, R.V.². 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE LINKÖPING, LINKÖPING - SUECIA.

Acute exercise results in accentuation of plasma cytokine levels and neutrophil mobilization. The capacity of these cells to respond to exogenous stimuli after exercise is still debated, as is the potential influence of nutritional factors. This study aimed to evaluate the function of neutrophils and cytokine production in individuals with varying dietary patterns following exhaustive isometric exercise. Healthy, trained men aged 19-44 years were categorized as omnivores (OMN), ovo-lacto-vegetarians (OLV), or strict vegetarians (SV) using the Food Frequency Questionnaire from ELSA-Brasil. Body composition was evaluated using dual-energy X-ray absorptiometry (DXA). Participants (n = 28; OMN = 9; OLV = 9; SV = 10) performed 24 sets of 10 maximal eccentric contractions on an isokinetic dynamometer. Blood samples were collected at pre, post, and 24 hours post-exercise. IL-6, IL-8, and IL-10 were measured by multiplex immunoassay. Neutrophil function was assessed in a subsample (n = 20; OMN = 7; OLV = 6; SV = 7) after 2 hours of LPS stimulation via phagocytosis and hydrogen peroxide (H₂O₂) production assays. No differences were observed between groups in terms of body composition, average peak torque (Nm), peak torque relative to body weight (Nm), total work (J), or work relative to body weight (J). IL-6 concentrations increased significantly over time (p = 0.002) from PRE (0.452 ± 0.346 pg/mL) to POST (0.706 ± 0.505 pg/mL), with a mean rise of 84.8% (± 108%). IL-10 levels showed a significant time effect with increases from PRE (0.227 ± 0.130 pg/mL) to 24H POST (0.310 ± 0.229 pg/mL) (42.7% ± 86.6%; pBonferroni = 0.011) and from POST (0.232 ± 0.156 pg/mL) to 24H POST (39.8% ± 66.9%; pBonferroni = 0.016). IL-8, phagocytic activity, and H₂O₂ production showed no significant differences. Acute resistance exercise influenced IL-6 and IL-10 responses independently of dietary pattern. Neutrophil function was not significantly affected by either exercise or dietary pattern. Keywords: Neutrophils; Exercise; Vegetarianism.

3052 - Category: CHEMOKINES AND TRAFFICKING (CT)

STUDY OF THE MECHANISMS INVOLVED IN THE MIGRATION AND INFILTRATION OF BLASTS IN T-CELL LYMPHOBLASTIC LEUKEMIA/LYMPHOMA

BELÓRIO, E.P.¹; DE JESUS, A.C.V.¹; CAMPOS, A.J.L.¹; FERNANDES-SANTOS, C.²; COTTA-DE-ALMEIDA, V.¹; MENDES-DA-CRUZ, D.A.¹. 1. IOC/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UFF, NOVA FRIBURGO - RJ - BRASIL.

INTRODUCTION: T-cell lymphoblastic leukemia/lymphoma (T-LLL) results from malignant proliferations of T precursors. In our recent *in vitro* assays, we found that sphingolipids, such as sphingosine-1-phosphate (S1P), and chemokines, such as CXCL12, can induce a chemotactic response in T-LLL blasts. The migration of T-LLL blasts is implicated in the infiltration of these cells into various organs, including the bone marrow and central nervous system (CNS), which are critically important for the prognosis of the disease. **OBJECTIVE:** To analyze the migration mechanisms of T-LLL blasts, including the exit from the thymus and invasion of the bone marrow and CNS. **RESULTS:** We analyzed the invasion capacity *in vitro* by inserting EL-4 (murine T-cell lymphoblastic lymphoma cell line) and Jurkat cells (human T-cell acute lymphoblastic leukemia cell line) into transwell chambers coated with MaxGel ECM. Our data showed that the invasion potential of EL-4 and Jurkat cells is stimulated under RPMI/FBS conditions. Furthermore, CXCL12 increased the invasion capacity of EL-4 cells under RPMI/FBS conditions and of Jurkat cells under both conditions. Additionally, the combination of CXCL12 and S1P further increased the invasive potential of EL-4 cells. However, blocking CXCR4 and S1P1 (the receptors for CXCL12 and S1P, respectively) did not reduce invasion. To standardize the *in vivo* model of T-LLL, 8-week-old male C57BL/6 mice were used. Our preliminary results showed an increase in thymus weight in these animals 7 days after intrathymic injections of EL-4 cells, compared with control animals. Histological analysis of the thymus revealed a disorganized structure, with no defined cortical and medullary regions, differing from the control group. **CONCLUSION:** Taken together, these data suggest the involvement of CXCL12 and S1P in the invasion of T-LLL blasts. The *in vivo* T-LLL model appears promising in elucidating the mechanisms of T-cell migration and invasion *in vivo*. **Keywords:** T-cell lymphoblastic leukemia/lymphoma ;Migration;Invasion.

3057 - Category: CHEMOKINES AND TRAFFICKING (CT)

THE ROLE OF SPHINGOSINE-1-PHOSPHATE, CXCL12, AND INTEGRINS IN TRANSENDOTHELIAL MIGRATION, INVASION, AND CHEMORESISTANCE OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOMA BLASTS.

DE JESUS, A.C.V.¹; LOIOLA, L.A.²; FERNANDES-SANTOS, C.²; BELÓRIO, E.P.¹; MENDES-DA-CRUZ, D.A.¹. 1. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL FLUMINENSE, NOVA FRIBURGO - RJ - BRASIL.

Introduction: T-cell lymphoblastic leukemia/lymphoma (T-LLL) is a neoplasia characterized by the proliferation of malignant T-cell progenitors, frequently infiltrating the bone marrow (BM) and central nervous system (CNS). Microenvironmental signals, such as sphingosine-1-phosphate (S1P) and the chemokine CXCL12, act through their respective receptors to mediate the migration and invasion of leukemic blasts. Additionally, extracellular matrix (ECM) components, such as fibronectin (FN), contribute to chemoresistance. This study aimed to investigate how these factors regulate T-LLL blasts infiltration and resistance to doxorubicin (DOXO) treatment. **Results:** Our results demonstrated that the combination of S1P and CXCL12 enhanced transendothelial migration of EL-4 cells, with no significant reversal upon receptor blockade. Rac1 inhibition reduced migration toward S1P, while co-inhibition with PI3K unexpectedly increased transmigration. Both stimuli also modulated the invasive capacity of the cells. Preliminary data suggest that S1P and CXCL12 confer protection against DOXO-induced apoptosis. **Conclusion:** Our data suggest a role for S1P and CXCL12 in the migration, invasion, and chemoresistance of T-LLL blasts. As next steps, we aim further to investigate the involvement of these molecules and glutamate, focusing on CNS and BM infiltration using *ex vivo* assays and the development of an *in vivo* T-LLL model. **Funding:** CNPq, FAPERJ, Fiocruz, CAPES, FOCEM and INCT-NIM. **Keywords:** T-cell lymphoblastic leukemia/lymphoma;Invasion ;Chemoresistance.

2646 - Category: CLINICAL IMMUNOLOGY (CL)

Evaluation of a chimeric recombinant protein-based ELISA for schistosomiasis diagnosis in endemic areas

FERNANDES, M.D.A.; BERNARDES, W.P.D.O.S.; DE CARVALHO, G.B.F.; PEREIRA, R.A.D.S.; FONSECA, C.T..
FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Diagnosing schistosomiasis remains challenging due to the growing number of low-intensity infections in endemic areas, which reduces the utility of the Kato-Katz method. As an alternative, the Enzyme-Linked Immunosorbent Assay (ELISA) has been considered; however, its low specificity limits its use in disease diagnosis. We hypothesize that incorporating a chimeric recombinant protein, comprising epitopes from multiple parasite antigens, into the ELISA would overcome this limitation. This study aimed to obtain a chimeric recombinant protein and to evaluate it in an ELISA-based test. A recombinant protein containing five epitopes from *Schistosoma mansoni* was engineered, expressed in a prokaryotic system, and purified via affinity chromatography. ELISA standardization involved selecting the microplate type, optimizing antigen and antibody concentrations, and blocking solutions. Sera from uninfected and infected individuals from an endemic area, previously characterized by several diagnostic methods, were used to evaluate the test's performance. The assay was conducted with two recombinant protein batches. An arbitrary unit (AU) was calculated using a calibrator in the assay. Data was analyzed using ROC curves to determine the cutoff value that maximized test accuracy. The recombinant protein (25kDa) was obtained with satisfactory purity and yield (4.9mg/L). The ELISA was performed using Maxisorp plates, coated with 12µg/mL of chimeric protein, and blocked with 3% BSA. Serum samples and HRP-conjugated anti-human IgG were used at 1:50 and 1:60.000 dilutions, respectively, with TMB as the substrate. A cutoff point of 0.18191 AU was applied for both batches. Batch 1 showed 76% sensitivity and 38% specificity % with an Area Under the curve (AUC) of 0.63; batch 2 showed 83% sensitivity and 30% specificity (AUC = 0.65). While the test showed high sensitivity, its low specificity limits its application to an initial screening of infected individuals in endemic areas. Keywords: Schistosomiasis;Diagnosis;Recombinant protein.

2663 - Category: CLINICAL IMMUNOLOGY (CL)

Development of a Monoclonal Antibody Targeting a Chimeric Monkeypox Virus Protein and Its Application in a Rapid Diagnostic Test Prototype

VALIATE, B.¹; LOPES, T.B.²; OLIVEIRA, J.K.A.²; DE BARROS, R.R.³; RAINER, M.N.D.²; FERNANDES, A.P.³; GAZZINELLI, R.T.¹. 1. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. CENTRO DE TECNOLOGIAS DE VACINAS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Monkeypox (Mpox), a zoonotic disease caused by the *Monkeypox* (MPXV) *virus*, has emerged as a global public health threat due to its rapid spread, severe morbidity, and potential for epidemic outbreaks. Unlike historical patterns, recent cases show human-to-human transmission beyond endemic regions, highlighting gaps in diagnostics and surveillance. The lack of accessible, rapid tests delays containment and treatment, exacerbating risks for vulnerable populations. Developing targeted diagnostics, like monoclonal antibody-based tests, is critical to curb transmission, enable early intervention, and prevent future outbreaks—especially in resource-limited settings where MPox is increasingly reported. Through hybridoma technology, we generated a monoclonal antibody targeting the chimeric protein ChimPox, which consists of the H3L and A29L proteins from the monkeypox virus (MPXV). To produce this antibody, BALB/c mice were immunized with purified ChimPox protein, followed by fusion of splenocytes with myeloma cells to create hybridomas. Subsequent screening and subcloning yielded a monoclonal antibody that demonstrates high specificity and binding affinity for the A29L domain of ChimPox. We employed this monoclonal antibody to develop an antigen-capture-based rapid test prototype for Mpox. Initial validation demonstrated high sensitivity and specificity when tested with MPXV samples. Current studies are evaluating the prototype using clinical specimens from Mpox patients, and preliminary results are promising. Beyond diagnostic applications, we plan to investigate the neutralizing capacity of this monoclonal antibody against MPXV through in vitro assays. Keywords: Monkeypox;Rapid test;monoclonal antibody.

2677 - Category: CLINICAL IMMUNOLOGY (CL)

INFLUENCE OF MATERNAL NUTRITIONAL STATUS ON THE INFLAMMATORY PROFILE OF HUMAN BREAST MILK

DE BARROS, H.L.P.¹; TORRES, L.¹; SILVA, I.M.E.¹; PEIXOTO, J.M.¹; DE CARVALHO, A.T.²; FARIA, A.M.C.¹; MAIOLI, T.U.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. INSTITUTO DE PESQUISA RENE RACHOU, BELO HORIZONTE - MG - BRASIL.

Human breast milk is a critical source of nutrition for newborns and infants, providing not only essential macronutrients and micronutrients necessary for early development but also immunological components that significantly contribute to the maturation of the infant immune system. Due to the dynamic nature of its composition, it is essential to monitor the nutritional status of lactating women, as maternal nutrition can influence the biochemical and immunological properties of breast milk. However, the extent to which maternal nutritional status affects the quality and inflammatory profile of breast milk remains poorly understood.

This study aimed to investigate the potential impact of maternal overweight and obesity on the immunological composition of breast milk during the early postpartum period. Lactating women between 20 and 90 days postpartum were stratified into two groups based on their pre-gestational Body Mass Index (BMI): a eutrophic group (normal BMI) and a group comprising overweight and obese individuals. The immune profile of milk samples was analysed using magnetic bead-based immunoassays performed on a Luminex platform.

The findings revealed that breast milk from overweight and obese mothers exhibited elevated levels of pro-inflammatory cytokines and chemokines, including IFN- α , IL-1 α , and MIP-1 α . Conversely, levels of cytokines with both pro- and anti-inflammatory functions, such as IL-3, IL-4, and IL-10, were reduced in this group. These results suggest that maternal nutritional status may influence the inflammatory milieu of breast milk in the first three months postpartum, potentially affecting neonatal immune development and health outcomes. Financial Support: FAPEMIG, CAPES and CNPq

Keywords: Human breast milk; Maternal nutritional status; Cytokine profile.

2682 - Category: CLINICAL IMMUNOLOGY (CL)

Food Allergy in Non-Obese Diabetic Mice (NOD)

SILVA, I.M.E.; DE BARROS, H.L.P.; MOREIRA, F.C.; PEIXOTO, J.M.; FARIA, A.M.C.; TORRES, L. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Administering low doses (0.1 μ g) of ovalbumin (OVA), a protein found in eggs, to mice can induce IgE production and long-lasting allergic responses in susceptible mice (BALB/c). However, little is known about whether mice with a genetic predisposition to other inflammatory diseases, such as type 1 diabetes mellitus (T1DM), can produce IgE and develop allergies. Recent studies show that non-obese diabetic (NOD) mice, which spontaneously develop T1DM, also exhibit changes in their gut microbiota and immune activity in the intestinal mucosa before the disease appears. Still, the connection between these alterations and food allergy development remains unclear. This study aimed to evaluate whether genetically predisposed animals are more susceptible to developing food allergies. We also investigated the allergen dose needed to produce specific IgE and the severity of allergic responses in NOD mice. To do this, we compared intraperitoneal immunization protocols using three different doses of OVA: low (1 μ g), standard (10 μ g), and high (100 μ g), in two mouse strains: NOD and BALB/c. After 14 days from the first immunization, a second dose was administered following the same protocol. Seven days later, mice underwent an oral challenge with a solution containing 30% egg white diluted in water. During this phase, we monitored body weight, solution intake, food consumption, and blood glucose levels. The results showed that NOD mice immunized with the high allergen dose (100 μ g) developed a more intense local allergic response, evidenced by Evans blue extravasation and increased total IgE levels. This indicates that, unlike what has been described for BALB/c mice, a high-dose allergen protocol effectively induced specific antibody production and an anaphylactic reaction after antigen challenge in NOD mice. Keywords: Food allergy; Type 1 diabetes mellitus; NOD mice.

2683 - Category: CLINICAL IMMUNOLOGY (CL)

Plasma Branched-Chain Amino Acids and Their Correlations with Inflammatory Markers

SOARES, B.F.S.¹; COELHO, M.M.¹; CAMPOLINA, B.G.¹; SCHNEIDER, F.S.²; PESSOA, C.A.¹; MOLINA, F.²; DUARTE, C.K.¹; MAIOLI, T.U.¹. 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. SYS2DIAG CNRS, MONTPELLIER - FRANÇA.

Recent in vitro studies have shown that elevated levels of branched-chain amino acids (BCAAs) can modulate immune cell polarization, particularly through mTORC1 activation. High BCAA concentrations may induce excessive reactive oxygen species (ROS) production, leading to mitochondrial dysfunction in peripheral blood mononuclear cells (PBMCs). Furthermore, BCAAs can activate NF-κB signalling, enhancing the secretion of pro-inflammatory cytokines and adhesion molecules.

Based on these findings, this study aimed to investigate the relationship between serum BCAA levels and inflammatory cytokines and chemokines in healthy volunteers. We analysed anthropometric data and plasma BCAA and inflammatory markers from healthy volunteers using the Luminex immunoassay technique employed to simultaneously quantify 48 cytokines and chemokines.

Our results demonstrated that significant negative correlations with BCAA levels were observed for beta-BGF, CCL27, IL-15, and TNF-α. Conversely, CXCL12, TRAIL, SCF, and CCL7 showed positive correlations. On the other hand, beta-BGF and VEGF presented higher levels in low BCAA plasma levels individual when compared with the higher ones. TNF-α and beta-BGF correlated negatively with HOMA-IR, while CXCL12 and CCL7 also correlated positively. Our findings suggest that BCAA blood concentrations are associated with specific inflammatory signatures. These results support the hypothesis that elevated BCAAs may influence immune modulation and systemic inflammation, warranting further investigation in our future larger cohort. Financial Support: Capes, CNPq, FAPEMIG, CNRS-France
Keywords: BCAA;Inflammation;HOMA-IR.

2710 - Category: CLINICAL IMMUNOLOGY (CL)

Association between biomarkers and executive functions in children and adolescents with obesity

FREITAS, B.A.D.A.¹; FREITAS, D.A.². 1. UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI, DIAMANTINA - MG - BRASIL; 2. UFVJM, DIAMANTINA - MG - BRASIL.

Obesity is a chronic disease characterized by excessive accumulation of fat in adipose tissue and has shown a concerning increase in prevalence among children and adolescents. It is associated with low-grade chronic inflammation and alterations in the secretion of biomarkers such as C-reactive protein (CRP) and cortisol. These mediators are linked to cardiometabolic diseases, stress, and cognitive decline, particularly through the release of systemic inflammatory cytokines. Considering the systemic effects of obesity, this study aimed to investigate the correlation between plasma levels of CRP and cortisol and the performance in executive functions among children and adolescents with obesity. This was a cross-sectional study including 32 participants aged between 7 and 18 years, all diagnosed with obesity. Executive functions were assessed using the Stroop Test, which evaluates inhibitory control, sustained, and selective attention. CRP levels were measured using immunoturbidimetry and cortisol levels using chemiluminescence in a clinical analysis laboratory. The mean age of participants was 11.25 ± 3.78 years, with an average body mass index (BMI) of 27.18 ± 3.78 kg/m². The mean CRP concentration was 4.15 ± 7.05 mg/L, and the mean cortisol level was 11.16 ± 5.03 mcg/dL. No significant correlation was found between CRP levels and Stroop Test performance ($p = 0.48$). However, a positive correlation was observed between cortisol levels and test performance ($p = 0.03$), suggesting that stress, rather than systemic inflammation, may influence executive function in this population. These findings highlight the importance of considering the role of chronic stress and its hormonal markers, such as cortisol, in the cognitive functioning of children and adolescents with obesity. We would like to thank FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) for their support in this study. Keywords: Obesity;cortisol;C-reactive protein.

2777 - Category: CLINICAL IMMUNOLOGY (CL)

e-Nature NK: A Randomized Controlled Trial on Nature, Immunity, and Mood

OLIVEIRA, L.P.G.¹; FIGUEIREDO, A.B.¹; DE OLIVEIRA, L.B.¹; SAVIETO, R.M.¹; BOMFIM, S.B.²; CATISSI, G.¹; GRAZIANI, J.B.¹; HINGST-ZAHER, E.³; LIMA, L.³; GOLLOB, K.J.¹; RIZZO, L.V.¹; LEÃO, E.R.¹. 1. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. INTERNATIONAL RESEARCH CENTER, HOSPITAL AC CAMARGO, SÃO PAULO - SP - BRASIL; 3. BIOLOGICAL MUSEUM, BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL.

Background: Emerging evidence suggests that contact with nature can modulate immune function through stress regulation and neuroendocrine pathways. This study aimed to compare the effects of direct and indirect nature exposure on mood states and immune parameters in people over 50 years old. **Methods:** Participants aged 50+ with physical and mental capacity were included. We conducted a parallel-group, non-masking randomized controlled trial with two groups: (1) direct exposure to a multicomponent nature-based intervention at the Butantan Institute's arboretum, and (2) indirect exposure involving a nature video composed of images from the e-Nature Positive Emotions Photography Database (e-NatPOEM), viewed at the Albert Einstein Research Center. Both lasted similar durations. Assessments included the Profile of Mood States (POMS), blood samples were collected for immune phenotyping by flow cytometry and a robust cytokine panel to evaluate systemic immune responses, conducted at baseline, 30 minutes, 48–72 hours, and 30 days post-intervention. **Findings:** Each group included 27 participants. Both groups showed improved overall POMS scores immediately post-intervention; however, the direct exposure group had significantly greater improvements in the fatigue-inertia and vigor-activity domains. In this group, there was a significant reduction in pro-inflammatory cytokines (IL-2, IL-6, and TNF- α) and Granzyme B, along with increased frequencies of CD8+ central memory and effector memory T cells ($p < 0.05$). A positive correlation was found between mood improvement and immune activation in the direct group ($r = 0.65$, $p = 0.002$). These findings suggest better mood and immune responses in the direct nature contact group, highlighting its therapeutic potential. **Keywords:** Nature based intervention; Lymphocyte response; Cytokine production.

2797 - Category: CLINICAL IMMUNOLOGY (CL)

Kidney tissue-derived extracellular vesicles as indicators of renal injury in pristane-induced lupus

MARTYRES, A.; ALVES, L.S.D.S.; VERÍCIMO, M.A.; AVELAR, T.M.T.; SILVA, A.A.. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL.

Pristane-induced lupus is an experimental model characterized by circulant autoantibodies and immune-complex glomerulonephritis. Extracellular vesicles (EVs) have been described in association with acute kidney disease, and may be isolated from the kidney (kEVs). We aimed to evaluate kEVs in pristane-induced lupus nephritis. This study was approved by the animal ethics committee (CEUA-UFF #471411022). Female BALB/c mice received a single intraperitoneal injection of pristane (0.5 mL; $n=21$) or saline ($n=14$). Kidney tissue, blood, and urine samples were collected at an early timepoint (T12 weeks) and at endpoint (T28 weeks) post-injection. We assessed kidney IgG deposition and plasma autoantibodies via indirect immunofluorescence. kEVs were obtained after tissue dissociation, isolated by differential centrifugation ($500\times g$ 5 min; $20,000\times g$ 20 min at $4^{\circ}C$), and characterized by transmission electron microscopy, nanoparticle tracking analysis (NTA), and nanoscale flow cytometry (nFC, CytoFLEX S). In nFC, EVs were gated by size (Gigamix beads, 100–900 nm) and Annexin V/podoplanin positivity. kEVs were normalized to kidney weight. IgG deposition and circulating autoantibodies were observed only in the pristane group at T28. At T12, the NTA showed higher mean EV diameter in pristane (162.8 ± 11.86 nm) compared to saline (127 ± 11.47 nm, $p=0.01$). Total kEV count by nFC was higher in pristane at T12 ($1.41E+08 \pm 2.98E+07/mL$ vs. $1.82E+07 \pm 5.74E+06/mL$, $p=0.041$) and at T28 ($8.39E+05 \pm 6.89E+05/mL$, $p<0.0001$). Podoplanin+ kEVs followed the same trend (T12: $2.12E+06 \pm 3.41E+05$ vs. T28: $3.83E+04 \pm 3.01E+04/mL$, $p<0.0001$). These findings demonstrate that counts of renal tissue-derived EVs are altered during the progression of experimentally-induced lupus nephritis, increasing even before the onset of glomerulonephritis. **Keywords:** Extracellular vesicles; Glomerulonephritis; Lupus.

2810 - Category: CLINICAL IMMUNOLOGY (CL)

PROTEOMIC ANALYSIS OF PLASMA EXTRACELLULAR VESICLES OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

LEMOS, A.P.D.A.¹; SAMPAIO, L.A.¹; GAUDIO, R.²; DA FONSECA, P.B.³; FELICE, F.³; NOGUEIRA, F.C.S.⁴; DOMONT, G.B.⁴; SILVA, A.A.⁵; AVELAR, T.M.T.⁶; CASTRO, M.J.C.⁵. 1. MULTIUSER LABORATORY FOR RESEARCH SUPPORT IN NEPHROLOGY AND MEDICAL SCIENCES (LAMAP), UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI, RIO DE JANEIRO, BRAZIL, NITERÓI - RJ - BRASIL; 2. REUMATOLOGY UNIT, HOSPITAL UNIVERSITÁRIO ANTÔNIO PEDRO, UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI, RIO DE JANEIRO, BRAZIL, NITERÓI - RJ - BRASIL; 3. D'OR INSTITUTE FOR RESEARCH AND EDUCATION (IDOR), RIO DE JANEIRO, BRAZIL., RIO DE JANEIRO - RJ - BRASIL; 4. PROTEOMICS UNIT, INSTITUTE OF CHEMISTRY AND PRECISION MEDICINE RESEARCH CENTER-IBCCF, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO - RJ - BRASIL; 5. DEPARTMENT OF PATHOLOGY, FACULTY OF MEDICINE, UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI, RIO DE JANEIRO, BRAZIL., NITERÓI - RJ - BRASIL; 6. DEPARTMENT OF PATHOLOGY, FACULTY OF MEDICINE, UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI, RIO DE JANEIRO, BRAZIL., NIT - RJ - BRASIL.

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease with great clinical heterogeneity and difficult clinical management. Since extracellular vesicles (EVs) are nanoparticles involved in inflammation and promising prognostic biomarkers in SLE, our goal was to develop a method for enriching plasma EVs (pEVs), depleting contaminants for proteomic analysis. In this cross-sectional study, plasma EVs from SLE patients were isolated by differential centrifugation, and PBS solution was used for additional washes. The protein profile of pEVs was evaluated by proteomic analysis. pEVs were quantified through nanoscale flow cytometry (nFC) and nanoparticle tracking analysis (NTA). We evaluated the effect of adding one or two pellet washes for EVs isolates. nFC and NTA showed a progressive decrease in the EV count in each wash (8% and 47%). A pool of three samples from SLE patients was evaluated by proteomic analysis, and we identified 332 proteins per single peptide. Of these, 70 were found exclusively in EVs, where 47% were of cytoplasmic origin and 22% nuclear. Washed EVs presented a different protein profile from plasma, and unwashed EVs presented a mixed profile. We observed an enrichment of EV markers, such as STOM and ITGB3, and proteins such as SAA1, an acute phase protein expressed in the liver, already mentioned as a possible biomarker for SLE. In the protein interaction analysis, we observed pathways related to hemolytic anemia, metabolism, cell organization and movement, endosomal regulation, platelet activation, as well as structural proteins of the ocular lens. Our data suggests that the EV enrichment methodology can improve the proteomic analysis of plasma EVs in SLE patients. We will apply the developed methodology to a panel of samples from SLE patients and non-SLE controls and evaluate the differences between the protein profiles obtained. Keywords: Proteomic analysis;systemic lupus erythematosus;extracellular vesicles.

2995 - Category: CLINICAL IMMUNOLOGY (CL)

A pilot study for the assessment of circulant and intravesicular VEGF in pediatric tuberculosis

CARVALHO, F.R.¹; NASCIMENTO, C.D.M.C.²; LEMOS, A.P.D.A.²; MARTYRES, A.²; CASTRO, M.J.C.²; AVELAR, T.M.T.²; SILVA, A.A.². 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITEROI - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL.

Diagnosing and managing tuberculosis (TB) remains a challenge in pediatric patients. Antigens secreted in response to *Mycobacterium tuberculosis* infection can lead to the secretion of extracellular vesicles (EVs), which are involved in defense mechanisms, especially in the immune response. It is known that VEGF plays an important role in the maintenance and pathophysiology of TB. Thus, our aim was to quantify circulant and intravesicular VEGF in pediatric TB and latent TB infection (LTBI). This cross-sectional study was approved by the Research Ethics Committee (CAAE: 70136023.0.0000.5243). TB/LTBI diagnoses were made using clinical, epidemiological, radiological, and immunological criteria. Serum EVs were isolated by differential centrifugation and characterized by transmission electron microscopy and nanoscale flow cytometry (CytoFLEX S) by size (Gigamix beads, 100–900 nm) and Annexin V positivity. VEGF was measured by ELISA in serum and lysed EVs. Samples from 16 pediatric patients (TB=8 and ILTB=8), in addition to seven healthy controls, with a mean age of 8 (± 4.9) years-old were analyzed. The majority was female (n=12) and of mixed race (n=12). Five patients were positive for Interferon-Gamma Release Assay and two for GeneXpert. We observed similar intravesicular VEGF levels in children and adolescents diagnosed with TB, LTBI, and healthy controls (0.128 pg/mL; 0.122 pg/mL and 0.127 pg/mL, $p > 0.05$). Surprisingly, we observed higher levels of intravesicular VEGF in TB and ILTB groups (TB: 0.13 pg/mL vs. 0.08 pg/mL, $p < 0.01$; LTBI: 0.12 pg/mL vs. 0.08 pg/mL, $p < 0.002$), which was not observed in the control group (0.01 vs. 0.01 $p = 0.16$). These findings indicate that quantification of intravesicular VEGF is feasible and reveal significant differences between TB-exposed individuals and may reflect the biological responses to *M. tuberculosis*, suggesting the potential of intravesicular VEGF as biomarker for pediatric TB. Keywords: extracellular vesicles;pediatric patients;Mycobacterium tuberculosis.

2998 - Category: CLINICAL IMMUNOLOGY (CL)

Association between NT-proBNP, GDF15, and IL-1 β in subclinical myocardial dysfunction in a cohort of individuals in the post-COVID-19 period

DO ROSÁRIO, N.F.; LOMBA, G.S.B.; RAMOS, A.; DE OLIVEIRA, E.M.; DE SOUZA, R.V.O.; CASTRO, M.J.C.; RIBEIRO, M.L.; DE CARVALHO, M.R.M.; LUGON, J.R.. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL.

Introduction: Persistent cardiac dysfunction following COVID-19 has been associated with activation of the immune and inflammatory systems, leading to inflammation-mediated myocardial injury. Activation of the interstitial mononuclear system may be a key factor in cardiac damage. Growth differentiation factor 15 (GDF15) is expressed in response to myocardial stress, as a cardioprotective factor and a remodeling indicator. The study analyzed the serum levels of GDF15, NT-proBNP, inflammatory cytokines and echocardiographic parameters in a cohort of patients in the post-COVID-19 period. **Methods:** This study is a cross-sectional analysis of data collected from 144 adult patients who had at least one positive PCR test for SARS-CoV-2. We identified a subgroup of participants who had elevated NT-proBNP levels at the initial laboratory evaluation (elevated NT-proBNP group; n=39). We also selected a control group from the same cohort with normal NT-proBNP levels at baseline (control NT-proBNP group), matched for sex, age, and the presence of comorbidities. Serum GDF15 and inflammatory mediators were quantified by immunoenzymatic and multiplex bead assays, respectively. Transthoracic echocardiography was performed using a commercial system. **Results:** The elevated NT-proBNP group had higher levels of GDF15 and IL-1 β , a higher rate of hospitalization for COVID-19 and reduced peak atrial longitudinal strain (PALS). The areas under the ROC curve for GDF15 and IL-1 β were 65.2% and 61.3%, respectively. The study cohort was stratified into elevated and normal GDF15 levels based on its cutoff value (519 pg/mL). Elevated GDF15 group had higher IL-8 levels and reduced right ventricular global longitudinal strain (RV GLS). **Conclusion:** Our results suggest that the association of NT-proBNP, GDF15, and IL-1 β could characterize subclinical myocardial dysfunction, reinforcing a pathophysiological model based on chronic inflammation, distinct from the classical ischemic paradigm. Keywords: post-COVID-19;GDF15;NT-proBNP.

3006 - Category: CLINICAL IMMUNOLOGY (CL)

Urinary podocyte extracellular vesicles and immune mediators as biomarkers in active lupus nephritis

AVELAR, T.M.T.¹; ALVES, L.S.D.S.¹; LEMOS, A.P.D.A.¹; GAUDIO, R.²; LIMA, H.F.¹; NASCIMENTO, C.D.M.C.¹; DA FONSECA, P.B.³; FELICE, F.³; LINO, K.²; RAMOS, A.¹; LACERDA, G.D.S.¹; CASTRO, M.J.C.¹; DE MATOS, J.P.S.¹; ALMEIDA, J.R.¹; BURGER, D.⁴; SILVA, A.A.¹. 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL; 2. HOSPITAL UNIVERSITÁRIO ANTONIO PEDRO, NITERÓI - RJ - BRASIL; 3. D'OR INSTITUTE FOR RESEARCH AND EDUCATION, RIO DE JANEIRO - RJ - BRASIL; 4. THE OTTAWA HOSPITAL RESEARCH INSTITUTE, OTTAWA - CANADA.

Urinary extracellular vesicles (uEVs) and immune mediators have emerged as potential minimally invasive renal biomarkers. Even though active lupus nephritis (LN) is associated with immune complex deposition, tissue inflammation, and podocyte damage, it remains unclear how these parameters are simultaneously altered in systemic lupus erythematosus (SLE). Thus, we aimed to evaluate uEVs as biomarkers in LN, in association with urinary immune mediators. In this cross-sectional study (Ethic Committee Approval #60747722.20000.5243), uEVs were isolated from SLE patients and healthy donors by differential centrifugation and assessed by transmission electron microscopy, nanoscale flow cytometry, and nanoparticle tracking analysis (NTA). Urinary immune mediators were assessed by a multiplex assay. We included 82 patients (42.6 $\pm$ 11.3 years-old, 91.4% female), of whom 56.1% (n=46) had LN, and 18 healthy donors (37.5 $\pm$ 8.2 years-old, 83.3% female). No differences were found for particle size/concentration by NTA, but higher counts of total (P=0.03) and podocyte-derived (P=0.01) uEVs were observed in SLE patients, especially in active LN (P=0.02; P=0.03). We also identified higher urinary levels of cytokines such as IL-6, IL-8, and CCL-2 according to SLE activity and LN (P<0.05). Significant correlations were observed between uEVs, immune mediators, R-SLEDAI-2K, proteinuria, and albuminuria in active LN. Lastly, the combinatory analysis of uEVs, uIL-8 and uCCL-2 showed a good predictive power to detect active LN (AUC=0.85, P=0.001). Our results suggest that urinary podocyte-derived uEVs and cytokines are associated with LN activity, which may reflect podocyte injury mediated by inflammation. Thus, the combined application these biomarkers could help to identify patients with podocyte damage and renal inflammation. Keywords: Extracellular vesicles;Systemic lupus erythematosus ;Urine.

3024 - Category: CLINICAL IMMUNOLOGY (CL)

EVALUATION OF THE EFFECTS OF THE ANESTHETICS SEVOFLURANE AND PROPOFOL ON THE PHENOTYPE AND FUNCTION OF MONOCYTE-DERIVED DENDRITIC CELLS

GUSTINELI, B.P.; BARBUTO, J.A.M.; VIEIRA, J.E.. USP, SÃO PAULO - SP - BRASIL.

Dendritic cells (DCs) play a crucial role in initiating and modulating immune responses. Among various factors influencing DC function, anesthetic agents have been shown to exert immunomodulatory effects, however they are still poorly understood. This study aims to evaluate the impact of two commonly used anesthetics - sevoflurane and propofol - on leukocytes populations and the phenotype and functional properties of monocyte-derived dendritic cells (moDCs). Human peripheral blood from patients undergoing minimal invasive surgery was collected at 3 times points (before anesthesia, 2h post-induction of anesthesia and 24h post-withdrawal of anesthesia) and flow cytometry of whole blood samples was performed to evaluate possible changes in leukocytes populations. The most significant finding was observed in subpopulations of monocytes where, despite the use of the same anesthetic, the frequency of Intermediate Monocytes (CD14⁺CD16⁺) decreased, while Non-Classical Monocytes (CD14⁻CD16⁺) increased over time. Furthermore, the monocytes were differentiated into DCs and cultured for 5 days and either activated (TNF- α , LPS, poli:IC) or maintained in its immature state. Phenotypic analysis was performed by flow cytometry to assess the expression of surface markers such as CD80, CD86, HLA-DR, and CD83. When comparing 2 patients that had surgery under the influence of propofol, a notable finding was a two-fold increase in the CD80/CD86 expression ratio of one of them, suggesting altered DC maturation even when using the same type of anesthetic. Preliminary findings indicate that anesthetics can alter leukocyte populations and lead to differential modulation of DC maturation, and thus, have implications for perioperative immune responses and postoperative outcomes. Further investigation is warranted to better understand these effects. This project is supported by FAPESP (2024/10411-2).
Keywords: Anesthesia; Monocyte-Derived Dendritic Cells; Immunomodulation.

3120 - Category: CLINICAL IMMUNOLOGY (CL)

NAÏVE REGULATORY T-CELLS AND IL-10 LEVELS CORRELATE WITH FAVORABLE CLINICAL OUTCOMES IN TYPE 1 DIABETES PATIENTS TREATED WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

DE SANTIS, P.B.¹; SANTANA-GONÇALVES, M.¹; DE FARIAS, K.C.R.M.²; RODRIGUES, M.C.D.O.¹. 1. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO - USP, RIBEIRÃO PRETO - SP - BRASIL.

Most Type 1 Diabetes (T1D) patients treated with Autologous Hematopoietic Stem Cell Transplantation (AHSCT) achieve insulin independence, remaining free of exogenous insulin for a median of 3 years. Mechanistic studies have suggested that favorable clinical outcomes are linked to improved immune tolerance and regulatory mechanisms. In this study, we investigated serum IL-10 levels and frequencies of regulatory T cells (Tregs) in the peripheral blood before and after AHSCT to better understand their association with clinical outcomes. Peripheral Blood Mononuclear Cells (PBMCs) from 9 T1D patients were analyzed by Flow Cytometry to identify total Tregs (CD3⁺CD4⁺CD25⁺FOXP3⁺) and naïve Tregs (CD3⁺CD4⁺CD25⁺FOXP3⁺CD45RA⁺). IL-10 levels were quantified by ELISA. Clinical outcomes were defined by the duration of insulin independence: short (<3 years) or long (>3 years). At 360 days post-transplant, patients with long-term insulin independence had significantly higher IL-10 levels ($p < 0.05$) (Figure 1). IL-10 concentrations positively correlated with C-peptide ($R^2 = 0.66$), suggesting preserved endogenous insulin production. Interestingly, IL-10 negatively correlated with total Treg frequencies but showed a strong positive correlation with naïve Tregs ($R^2 = 0.70$), which increased significantly by day 100 post-AHSCT ($p < 0.05$) (Figure 2). Naïve Tregs also correlated with C-peptide ($R^2 = 0.43$) (Figure 3). These results suggest that naïve Tregs may be a major source of IL-10, which in turn is associated with improved beta-cell preservation and function. Moreover, patients with better outcomes consistently exhibited higher IL-10 levels across all time points. In conclusion, increased IL-10 concentrations and expansion of naïve Treg populations were found to be associated with favorable clinical outcomes in T1D patients treated with AHSCT, indicating a potential immunoregulatory pathway that supports long-term insulin independence and beta-cell integrity.
Keywords: Type 1 Diabetes; Autologous Stem Cell Transplantation; Immunoregulation.

3136 - Category: CLINICAL IMMUNOLOGY (CL)

DETECTION OF PLASMA EXTRACELLULAR VESICLES BY AN IN-HOUSE IMMUNOENZYMATIC METHOD AS A POTENTIAL MONITORING TOOL FOR SYSTEMIC LUPUS ERYTHEMATOSUS

SAMPAIO, L.A.¹; LEMOS, A.P.D.A.²; GAUDIO, R.³; AVELAR, T.M.T.⁴; SILVA, A.A.⁴; CASTRO, M.J.C.⁴. 1. UNDERGRADUATE IN BIOMEDICINE, UNIVERSIDADE FEDERAL FLUMINENSE (UFF), NITERÓI, RIO DE JANEIRO, BRAZIL, NITERÓI - RJ - BRASIL; 2. MULTIUSER LABORATORY FOR RESEARCH SUPPORT IN NEPHROLOGY AND MEDICAL SCIENCES (LAMAP) AND GRADUATE PROGRAM IN PATHOLOGY; FACULTY OF MEDICINE, UNIVERSIDADE FEDERAL FLUMINENSE (UFF), NITERÓI - RJ - BRASIL; 3. RHEUMATOLOGY UNIT, HOSPITAL UNIVERSITÁRIO ANTÔNIO PEDRO, UNIVERSIDADE FEDERAL FLUMINENSE (UFF), NITERÓI - RJ - BRASIL; 4. DEPARTMENT OF PATHOLOGY AND GRADUATE PROGRAM IN PATHOLOGY; FACULTY OF MEDICINE, UNIVERSIDADE FEDERAL FLUMINENSE (UFF), NITERÓI - RJ - BRASIL.

Systemic lupus erythematosus (SLE) is a complex autoimmune disease whose clinical heterogeneity makes diagnosis and monitoring challenging, and the extracellular vesicles (EVs) emerge in this scenario as promising biomarkers. This study aims to evaluate the potential of plasma EVs as a monitoring biomarker for SLE using an in-house enzyme-linked immunosorbent assay (ELISA). We analyzed 30 plasma samples from SLE patients, stratified according to the disease clinical manifestation, and 10 samples from non-SLE individuals as a control group. The EVs were isolated from plasma samples using differential centrifugation and washed with phosphate-buffered saline (PBS) to reduce potential plasma interferents. The in-house ELISA methodology was previously standardized for detecting platelet-derived EVs, where we defined the best dilution to be applied to these previously isolated EVs samples. After standardization, the samples were diluted 1/10.000, and the groups of SLE patients and controls were evaluated by analyzing their respective optical densities (OD). The results demonstrated that the group with renal involvement had a significantly higher OD when compared to the cutaneous-articular group ($p = 0.02$) and the cutaneous-articular + hematologic group ($p = 0.03$). No significant differences were observed among the groups with other clinical manifestations of SLE. However, patients with some renal involvement showed higher detection of EVs than patients without renal involvement ($p = 0.02$). A new panel of samples will be evaluated using the previously standardized methodology. These results suggest that detection of platelet-derived EVs by ELISA may be an effective tool for monitoring SLE, with potential applicability in laboratory routine, since this technique has a well-established application structure in clínica-laboratorial practice.

Keywords: Extracellular vesicles;systemic lupus erythematosus;enzyme-linked immunosorbent assay.

3165 - Category: CLINICAL IMMUNOLOGY (CL)

OBESITY AS AN IMMUNOMETABOLIC RISK FACTOR IN COVID-19: INTEGRATED ANALYSIS OF PLASMA PROFILES OF TNF, IL-6, IL-10, AND RESISTIN IN DIFFERENT CLINICAL OUTCOMES

OTONI, C.D.M.¹; QUEIROZ, W.D.M.¹; REIS, A.P.G.¹; PIMENTA, A.L.B.¹; SILVA, K.O.¹; SILVA, R.D.J.¹; MIRANDA, L.V.D.J.¹; CAMPOS, C.I.A.¹; GARCIA, B.C.C.¹; CARNIO, E.C.²; VIEIRA, E.R.¹; COSTA, M.L.B.¹; FIGUEIREDO, V.G.¹; SANTOS, J.D.¹; RODRIGUES, C.M.¹. 1. UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI, DIAMANTINA - MG - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Obesity has been identified as an important risk factor for COVID-19, especially due to its chronic inflammatory nature and its role in immune dysregulation. This cross-sectional cohort study aimed to evaluate the influence of obesity on plasma levels of TNF, IL-6, IL-10, and resistin cytokines in individuals infected with SARS-CoV-2, correlating these biomarkers with the clinical severity of the infection. A total of 142 adults who tested positive for SARS-CoV-2 by RT-PCR were included, classified as having flu-like syndrome (FLS) and severe acute respiratory syndrome (SARS), in addition to the negative control group. Participants were stratified according to body mass index (BMI) into eutrophic and obese groups. Plasma concentrations of the markers were measured by ELISA. Statistical analysis was performed in GraphPad Prism using the Two-Way ANOVA test followed by Tukey's post hoc test ($p < 0.05$). The results revealed that obese individuals had elevated levels of TNF and IL-6 in both SG ($p = 0.03$ and $p < 0.0001$, respectively) and SARS ($p = 0.02$ and $p < 0.0001$, respectively). IL-10, in turn, showed a reduction in individuals with SG ($p = 0.0001$). In cases of SRAG, there was also a reduction, but without statistical significance ($p = 0.07$). When comparing individuals with and without obesity within the SG and SARS groups, it was observed that patients with obesity had lower levels of IL-10. However, when analyzing only within each group (intragroup), this difference between BMI subgroups was not statistically significant. Regarding resistin, there was no statistical difference. These findings indicate an immune imbalance characterized by an increase in mediators associated with the inflammatory response. This scenario favors the intensification of the immune response, contributing to the cytokine storm, lung injury, and worsening of the clinical condition. Obesity, therefore, can compromise immune regulation and should be considered a critical factor in COVID-19. Keywords: Immunometabolic obesity;Clinical severity of COVID-19;Plasma cytokines.

3182 - Category: CLINICAL IMMUNOLOGY (CL)

Serological and functional evaluation of *Blomia tropicalis* and *Dermatophagoides pteronyssinus* allergens in allergic patients as potential allergy biomarkers

SOUSA, J.E.A.¹; ORRICO-FERREIRA, C.M.¹; RIOS, J.V.B.²; CRUZ, P.E.D.O.²; SOUZA, L.M.¹; SENA, N.C.²; MELO, V.L.M.²; DE ANDRADE, J.C.D.C.¹; SILVA, R.C.¹; DA SILVA, E.S.¹; JAQUECT, A.³; CHANASIT, S.³; PINHEIRO, C.D.S.³. 1. PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA –PPGIM, SALVADOR - BA - BRASIL; 2. UNIVERSIDADE FEDERAL DA BAHIA - UFBA, SALVADOR - BA - BRASIL; 3. DEPARTMENT OF BIOCHEMISTRY, FACULTY OF MEDICINE, CHULALONGKORN UNIVERSITY, BANGKOK, THAILAND, THAILAND - TAILANDIA.

Introduction: House dust mite (HDM) allergy affects 1–2% of the global population, with high treatment costs and morbidity. Key species like *Blomia tropicalis* and *Dermatophagoides pteronyssinus* drive sensitization across demographics, despite the high prevalence of HDM allergy, no molecular panels exist to predict asthma severity. Characterizing their allergens is vital for clinical advances and precision diagnostic tools. **Objective:** Develop a recombinant HDM allergen panel as potential biomarkers for allergic asthma severity. **Material and Methods:** Seven recombinant allergens (rBlo t 1, 2, 5, 21, 41, rDer p 5, 20, 21) were successfully expressed and purified. Their IgE reactivity was assessed by indirect ELISA using sera from allergic patients (n° 25000.013834/2010-96). Additionally, the biochemical characterization of rBlo t 41 was performed for the first time since its identification as an allergen. **Results:** The chitin-binding assay confirmed that rBlo t 41 is a functional chitin-binding protein. In serological analysis, rBlo t 41 showed higher IgE reactivity in allergic rhinitis (59%) vs. allergic asthma (18%). Blo t 2, 5, 21 and Der p 5, 21 had >50% IgE reactivity. Six allergens (Blo t 2, 5, 21, Der p 5, 20, 21) showed strong correlation with the HDM extracts. Blo t 2, 5, and 21 showed significant correlation with the severity of allergic asthma (Fisher's exact test, p<0.05). **Conclusion:** This study highlights the potential of recombinant allergens as precise tools for allergy diagnosis and disease stratification. Blo t 2, 5, and 21 not only showed strong correlation with house dust mite extracts but also with asthma severity. These findings contrast with previous extract-based approaches, suggesting that selected recombinant allergens may serve as reliable biomarkers for identifying disease phenotypes and stratifying asthma severity. This supports their potential use in diagnostic panels for personalized therapies.

Keywords: House dust mite;Allergy;Molecular painel.

3187 - Category: CLINICAL IMMUNOLOGY (CL)

CLINICAL, EPIDEMIOLOGICAL, AND VIRAL GENOME CHARACTERISTICS OF CHILDREN HOSPITALIZED FOR RESPIRATORY SYNCYTIAL VIRUS INFECTION AT A SENTINEL UNIT FROM 2018 TO 2024

FACCION, R.S.¹; DE SOUZA, L.D.R.¹; DA COSTA, A.C.C.¹; LOPES, M.¹; PIAZI, M.D.¹; REIS, L.²; PEREIRA, E.C.²; BENTO, L.P.F.²; SIQUEIRA, M.M.²; RESENDE, P.C.²; VASCONCELOS, Z.¹. 1. IFF/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. IOC/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Objective: To investigate clinical-epidemiological characteristics and genomics of the virus and of the children hospitalized with Respiratory Syncytial Virus (RSV) from 2018 to 2024 at *Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira/Fiocruz*. **Methods:** This is a descriptive, cross-sectional, and retrospective study. Clinical and epidemiological data were analyzed using softwares SPSS (v.2022) and R (v.4.3.1). Nasopharyngeal samples were scanned for viruses using the BD Max system. **Results:** Of a total of 1,187 cases of severe acute respiratory infection (SARI) due to respiratory viruses admitted to this hospital, 198 RSV-positive cases fulfilled the study's inclusion/exclusion criteria. The age range of 0-3 months and male gender were predominant in the three periods studied (before, during, and after the pandemic). The most frequently reported symptoms at the onset of infection were respiratory distress, cough, fever, and oxygen saturation <95%. There was no change in the frequency of admission or length of stay in intensive care, although the total length of stay decreased over the years. We were able to recover 114 viral nasopharyngeal samples, and sequenced 40 samples so far. The most frequent clades were A.D.1.4 (12 events), peaking in November 2020, and B.D.E.1 (9 events), peaking in May 2024, which was compatible with clades' circulation in Brazil in the period. **Conclusion:** So far, our data indicates that management of children with SARS due to RSV improved over the years, with a reduction of total length of hospitalization in our hospital. The next steps are to investigate possible correlations between clinical features, viral clade and patient's genetics, especially possible inborn errors of immunity-related findings that can account for the infection severity in these children.

Keywords: Respiratory Syncytial Virus;Metagenomic surveillance;Inborn Errors of Immunity.

3267 - Category: CLINICAL IMMUNOLOGY (CL)

VITA MONITORING: LONGITUDINAL ANALYSIS OF COMPLETE BLOOD COUNT, D-DIMER, FERRITIN, CRP AND HS-CRP IN INDIVIDUALS WITH POST-COVID CONDITIONS AT 3 AND 6 MONTHS, AND THEIR CLINICAL CORRELATION.

BARRA, M.R.D.P.¹; SODRE, S.¹; LIMA, A.E.D.S.¹; LOURENÇO, A.J.¹; FILGUEIRAS, P.S.¹; OLIVEIRA, L.A.R.¹; CONTIN, S.V.M.¹; CAMARGOS, I.R.¹; MAGALHÃES, V.R.M.¹; DA SILVA, L.G.¹; CORRÊA, P.R.L.²; GOMES, M.F.D.C.³; QUEIROZ, R.F.G.E.¹. 1. INSTITUTO RENE RACHOU - FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. MINISTÉRIO DA SAÚDE, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSITY OF GEORGIA, GEORGIA - ESTADOS UNIDOS DA AMERICA.

Post-covid conditions involve symptoms that arise or persist for more than four weeks after infection with SARS-CoV-2, without any other identifiable cause. Despite their impact on the functionality of affected individuals, the mechanisms involved are not yet fully elucidated, and the lack of specific diagnostic tests makes diagnosis primarily clinical. The objective of this study is to evaluate the production of inflammatory and injury biomarkers — such as C-reactive protein (CRP), both conventional and highsensitivity (hsCRP), ferritin, D-dimer, and blood count parameters — at 3 and 6 months in individuals with post-covid conditions, focusing on therapeutic response and clinical progression. This observational subproject is part of the VITA Monitoring study, which recruited 212 individuals living in Belo Horizonte/MG with persistent symptoms in the post-covid context. Most participants were adult women, with a mean age of 47 years. In-person visits were conducted at an initial moment, 1, 3, and 6 months and included medical evaluation, structured questionnaires, and blood sample collection for laboratory tests, including immunochromatographic assays and complete blood count. Preliminary results suggest an improvement in the inflammatory profile, with significant differences observed between baseline and the 6-month follow-up, including a decrease in the following markers: D-dimer (237 ng/mL; $p < 0.0001$), hemoglobin and hematocrit ($p = 0.01$), red blood cells and basophils ($p = 0.04$). These findings suggest potential changes related to therapeutic response or disease progression. The outcomes contribute to identifying useful markers in the clinical follow-up of patients with post-covid conditions and may support the development of care protocols and public health policies aimed at addressing them. New assays are underway and are expected to help clarify the findings and strengthen the scientific evidence on the subject.

Keywords: POST-COVID CONDITIONS; SARS-COV-2; BIOMARKERS.

3268 - Category: CLINICAL IMMUNOLOGY (CL)

Identification of Genetic Variants in Hemophagocytic Lymphohistiocytosis and Predisposition to Childhood Acute Lymphoblastic Leukemia

MORGADO, E.S.¹; AGONIGI, B.N.D.S.¹; CUNHA, D.P.¹; FACCION, R.S.¹; POMBO-DE-OLIVEIRA, M.S.²; DE VASCONCELOS, Z.F.M.¹. 1. INSTITUTO NACIONAL DE SAÚDE DA MULHER, DA CRIANÇA E DO ADOLESCENTE FERNANDES FIGUEIRA (IFF/FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer, with multifactorial etiology involving genetic predisposition, immune dysregulation, and environmental triggers. Inborn Errors of Immunity (IEI), particularly those related to Hemophagocytic Lymphohistiocytosis (HLH), have been associated with impaired cytotoxic function and may contribute to leukemogenesis. This study aimed to investigate the presence of pathogenic variants in HLH-related genes in children with ALL through whole-exome sequencing (WES). We analyzed 29 samples (23 B-ALL and 6 T-ALL) from two cohorts: EMiLI (INCA) and RENOMIEII (IFF/Fiocruz). DNA was extracted and sequenced using the Illumina NovaSeq 6000 platform with PCR-Free Library Prep. Reads were processed using the nf-core Sarek pipeline, aligned to the hg38 reference genome, and variant calling was performed with HaplotypeCaller. VCF files were uploaded to the Franklin (Genoox) platform for sequencing quality control and variant annotation. Variants were classified according to ACMG 2023 guidelines, prioritizing rare ($\leq 1\%$) non-silent SNVs and INDELs in coding or splicing regions. A custom LLA-HLH gene panel was applied, covering genes involved in lymphocyte cytotoxicity, macrophage activation, antiviral defense, autophagy, inflammasome regulation, metabolism, Th1/Th2 balance, and recurrently mutated genes in B-/T-cell leukemias and lymphomas. Among 17 cases with variants of interest, 37 variants were identified: 2 pathogenic and 9 likely pathogenic (7 frameshift, 3 non-synonymous, 1 non-frameshift), all in heterozygosity. Notably, PRF1 and RAG1 variants were found, both previously linked to HLH. Further analyses including WGS and additional samples are ongoing. Keywords: Acute Lymphoblastic Leukemia; Inborn Error of Immunity; Hemophagocytic Lymphohistiocytosis.

3297 - Category: CLINICAL IMMUNOLOGY (CL)

Immunoinflammatory profile in substance users with and without psychiatric diagnosis

DUARTE, F.O.¹; CAMILLO, L.¹; BIANCHI, J.¹; DE GODOY, K.F.¹; RODOLPHO, J.M.D.A.¹; FRAGELLI, B.D.D.L.¹; SPEGLICH, C.²; PRADO, J.D.A.¹; ANIBAL, F.¹. 1. UFSCAR, SÃO CARLOS - SP - BRASIL; 2. PETROBRAS, RIO DE JANEIRO - RJ - BRASIL.

Evidence suggests a relationship between alcohol and/or drug use and mental disorders, although the biological mechanisms associated with depression remain incompletely understood. Prolonged stress and anxiety may dysregulate the hypothalamic-pituitary-adrenal axis, increasing cortisol and pro-inflammatory cytokine levels, potentially contributing to neuroinflammation and the development of psychiatric disorders. This study aimed to compare levels of cortisol, CRP, IL-6, IL-8, IL-1 β , TNF and vitamin D among healthy volunteers and patients with substance use, with and without psychiatric diagnoses. 256 volunteers were included, comprising 122 in control group and 134 hospitalized for substance abuse. Serum samples were analyzed using immunofluorescence and chemiluminescence. Statistical analysis was performed using GraphPad Prism software, applying the Kruskal-Wallis test followed by Dunn's post hoc test, with significance $p < 0.05$. Results showed significant increase in cortisol levels in both substance user groups ($p < 0.001$), indicating activation of hypothalamic-pituitary-adrenal axis. CRP was elevated only in group without a psychiatric diagnosis ($p < 0.0001$). Both IL-6 ($p < 0.0001$) and IL-1 β ($p < 0.05$) were increased in both user groups while IL-8 decreased in non-psychiatric group ($p < 0.001$). TNF levels were reduced in psychiatric group ($p < 0.05$). No significant differences were observed in vitamin D levels. These findings indicate inflammatory and neuroendocrine alterations associated with substance use, with distinct patterns depending on the presence of psychiatric comorbidity. In addition to inflammation, the observed changes reflect potential immune imbalances, suggesting impaired regulation of immune responses in these individuals. The identification of biomarkers may contribute to the refinement of integrated diagnostic and therapeutic strategies targeting the mental health of populations vulnerable to substance use. **Funding:** PETROBRAS 2022/00220-0. **Keywords:** Substance abuse; Cytokines; Mental disorders.

3332 - Category: CLINICAL IMMUNOLOGY (CL)

Large-scale Manufacturing of CD19 CAR-T cells using a minicircle-based non-viral DNA vector carrying a Sleeping Beauty transposon platform

DOURADO, L.P.S.¹; SILVA, L.R.B.¹; DOS REIS, S.A.¹; ABDO, L.D.M.¹; ARAGAO, E.A.A.¹; BONAMINO, M.H.². 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. INCA/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Immunotherapy with CD19-directed chimeric antigen receptor T cells (CAR-T) has shown high response rates in B-cell malignancies. However, current CAR-T manufacturing relies on retroviral or lentiviral vectors, which are costly and complex, limiting its broader use. To overcome these barriers, we employed a non-viral minicircle (MC) plasmid system based on the Sleeping Beauty (SB) transposon to generate anti-CD19 CAR-T cells. MCs are derived from parental plasmids processed in bacteria expressing inducible recombinase, resulting in vectors containing only the therapeutic transgene under a specific promoter, free of prokaryotic sequences—enhancing safety and efficiency.

Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated and enriched for T cells via CD3+ magnetic bead selection. Cells were activated for 48h and nucleofected using the 4D-Nucleofector System (Lonza), LV Unit, with the MC PT4 19BBz vector and SB100x transposase. Cultures were expanded using the G-Rex system, Wilson Wolf's gas-permeable membrane technology, in the presence of the cytokine IL-2.

In preliminary small-scale results, the system showed promising results; thus, we now present the efficacy and safety of the system also on a large scale. The results showed high viability (>90%) and CAR expression (>30%) after 6 days. From 5×10^7 T cells, we obtained over 1×10^8 CAR-T cells. In vivo studies in NSG mice bearing Nalm-6 leukemia demonstrated dose-dependent antitumor activity. Mice treated with 5×10^6 CAR-T cells showed noticeably improved survival compared to those that received 1×10^6 cells, and significantly greater survival compared to untreated controls ($p=0.01$).

In conclusion, this virus-free platform enabled efficient large-scale CAR-T cell production with robust antitumor activity, offering a scalable and cost-effective alternative to viral vector-based methods.

Keywords: CAR-T; Transposon; Minicircle.

3336 - Category: CLINICAL IMMUNOLOGY (CL)

Development of a Graphene Oxide-Functionalized Long-Period Fiber Grating Biosensor for Selective Detection of Interleukin-1 β

SOARES, S.M.A.; DO COUTO, M.S.; DE QUEIROZ, L.H.; SILVA, D.B.D.F.; FERREIRA, J.P.E.; WATANABE, A.S.A.; QUIRINO, W.G.; SANTOS, M.D.O.; GAMEIRO, J.. UFJF, JUIZ DE FORA - MG - BRASIL.

Cytokine detection is commonly based on antigen–antibody interactions, typically employing immunoassays. Although these methods are widely adopted, they remain time-consuming and costly, motivating the pursuit of more efficient alternatives. In this context, highly sensitive and selective biosensors have emerged as promising tools to advance biomedical diagnostics. This study reports the fabrication and characterization of an optical biosensor based on Long-Period Fiber Gratings (LPG), designed specifically to detect the pro-inflammatory cytokine interleukin-1 β (IL-1 β). The sensor surface was functionalized with graphene oxide (GO), which provides a highly efficient platform for bioaffinity interactions, while anti-IL-1 β antibodies were covalently immobilized via EDC/NHS activation chemistry to ensure stable binding. To enable testing in liquid media, the biosensor was encapsulated in a microfluidic capsule. Surface characterization by AFM, SEM, and Raman spectroscopy confirmed successful GO deposition and effective biofunctionalization. Spectral analysis revealed systematic resonance wavelength shifts correlating with increasing IL-1 β concentrations, achieving a detection limit of 14.9 pg/mL. Additionally, the device demonstrated excellent selectivity, showing no similar response to the non-target cytokine IL-10. Collectively, these findings show the potential of the GO-functionalized LPG biosensor as a rapid, sensitive, and cost-effective platform for clinical diagnostics, with versatility for detecting other biomarkers and integration into multiplexed detection systems.

Keywords: Biosensor;Diagnostic;Interleukin-1 β .

3381 - Category: CLINICAL IMMUNOLOGY (CL)

Correlation between MMPs 2 e 8 levels and inflammatory cytokines in patients with Inflammatory Bowel Diseases

NETO, P.V.D.S.¹; DOS SANTOS, M.M.¹; SILVA, M.D.¹; NETO, F.M.D.S.²; LUCAS, J.M.³; PARRA, R.S.⁴; FEITOSA, M.R.⁵; FERES, O.⁵; DA ROCHA, J.J.R.⁶; SORGI, C.A.⁷; CARDOSO, C.R.D.B.¹. 1. DEPARTAMENTO DE ANÁLISES CLÍNICAS, TOXICOLÓGICAS E BROMATOLÓGICAS, FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO - FCFRP/USP, BRAZIL, RIBEIRÃO PRETO - SP - BRASIL; 2. 1DEPARTAMENTO DE ANÁLISES CLÍNICAS, TOXICOLÓGICAS E BROMATOLÓGICAS, FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO - FCFRP/USP, BRAZIL, RIBEIRÃO PRETO - SP - BRASIL; 3. DEPARTAMENTO DE BIOQUÍMICA E IMUNOLOGIA, FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - FMRP/USP, BRAZIL, RIBEIRÃO PRETO - SP - BRASIL; 4. DEPARTAMENTO DE CIRURGIA E ANATOMIA DA FACULDADE DE MEDICINA DE RIBEIRÃO PRETO DA UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO, SP, BRAZIL., RIBEIRÃO PRETO - SP - BRASIL; 5. DEPARTAMENTO DE CIRURGIA E ANATOMIA DA FACULDADE DE MEDICINA DE RIBEIRÃO PRETO DA UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO, SP, BRAZIL, RIBEIRÃO PRETO - SP - BRASIL; 6. DEPARTAMENTO DE BIOQUÍMICA E IMUNOLOGIA, FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - FMRP/USP, BRAZIL., RIBEIRÃO PRETO - SP - BRASIL; 7. FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO, SP, BRAZIL., RIBEIRÃO PRETO - SP - BRASIL.

There is a clear correlation between immune dysregulation in inflammatory bowel diseases (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC). Matrix metalloproteinases (MMPs) contribute to tissue destruction in many pathological situations, and MMP activity leads to the release of bioactive mediators with inflammatory properties. We sought to characterize a scenario in which MMPs could influence the pathogenesis of IBD. Thus, our objective here was to quantify MMPs (8 and 2) and inflammatory cytokines in patients with CD and UC, to correlate with the patient's inflammatory response and the clinical presentation of the disease. Sixty-five individuals (53 with CD and 12 with UC) and 60 healthy controls were included in this study and had plasma samples tested for cytokines (IL-6, IL-10, IFN, TNF- α) and MMPs 2 and 8, in addition to clinical evaluation. Patients with IBD showed increased MMP-8 and MMP-2, especially those with colonic and fistulizing CD. Furthermore, CD patients had elevated plasma IL-6 levels. There was a positive correlation between MMP-8 and circulating LPS levels in CD in patients with total IBD. Furthermore, IL-6 and MMP-8 levels were correlated in CD, indicating an influence by the inflammatory response. Finally, overexpression of MMP-2 and MMP-8 may be considered relevant biomarkers of CD and IBD presentation.

Keywords: Crohn's disease;MMPs;disease activity.

3445 - Category: CLINICAL IMMUNOLOGY (CL)

High CCL5 Levels and IL-4/IL-17 Correlations Reveal Systemic Inflammation in Long COVID Olfactory Dysfunction

DOS SANTOS, J.C.¹; DE CARVALHO, D.M.²; RABELO, M.X.³; DE CARVALHO, L.I.A.⁴; ALVES, T.C.D.L.⁵; FUJIMOTO, V.C.M.⁵; PONTES, G.S.¹. 1. PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA BÁSICA E APLICADA - PPGIBA/UFAM, MANAUS - AM - BRASIL; 2. UNIVERSIDADE FEDERAL DO AMAZONAS / PPGIBA, MANAUS - AM - BRASIL; 3. CENTRO MULTIUSUÁRIO PARA ANÁLISE DE FENÔMENOS BIOMÉDICOS DA UNIVERSIDADE DO ESTADO DO AMAZONAS - CMABIO, MANAUS - AM - BRASIL; 4. UNIVERSIDADE DO ESTADO DO AMAZONAS - UEA, MANAUS - AM - BRASIL; 5. UNIVERSIDADE FEDERAL DO AMAZONAS - UFAM, MANAUS - AM - BRASIL.

The persistence of symptoms for more than 3 months after the onset of acute COVID-19 characterizes Long COVID, a complex and multisystemic condition that still remains poorly understood. The aim of this study was to evaluate the clinical-epidemiological characteristics and the immune profile of patients with Long COVID characterized by persistent olfactory dysfunction for more than 6 months. The study included 60 adult patients diagnosed with Long COVID and 20 healthy individuals. Each participant completed a clinical-epidemiological questionnaire and underwent a comprehensive otorhinolaryngological examination, including nasal videoendoscopy and psychophysical olfactory testing. Additionally, blood samples were collected from all participants for cytokine profile analysis. Predominantly, both groups consisted of women, with a higher prevalence of pre-existing comorbidities observed in the Long COVID group. Moreover, signs of nasal inflammation were more frequently detected during nasal videoendoscopy in Long COVID patients compared to the healthy control group. The analysis of cytokine profiles revealed a significant increase in pro-inflammatory cytokines, suggesting an acute and dysregulated inflammatory state among the group with Long COVID. The chemokine CCL5 was identified as the marker with the highest serum levels. Furthermore, nine strong positive correlations were observed among immune markers, with the strongest correlation found between IL-4 and IL-17. These findings indicate that nasal mucosal inflammation in Long COVID patients is associated with a persistent inflammatory state, as evidenced by the systemic inflammatory profile of these individuals. This highlights the need to better understand the underlying immunological mechanisms to improve the clinical management of these patients.

Keywords: Long COVID;olfaction disorders;inflammation.

3471 - Category: CLINICAL IMMUNOLOGY (CL)

Association between pesticide exposure and cytokines alterations in a rural population of Teresópolis, Rio de Janeiro, Brazil

MOTA, C.D.L.; DACRI, I.M.; ESPINDOLA, A.D.S.; DA COSTA, S.P.; VIANNA, M.S.; TORRES, J.P.M.; LIMA, J.E.N.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Exposure to environmental contaminants, such as pesticides, results in many health problems. Agricultural workers have significantly higher exposure, representing a significant Public Health problem. An increasing number of studies have identified an association between pesticide exposure and adverse effects on the immune system; however, the mechanisms remain unknown. OBJECTIVE: This study aimed to investigate the impact of exposure to pesticides on the modulation of cytokines in a sample of the population from Teresópolis city, one of the main agricultural areas of Rio de Janeiro, Brazil. METHODOLOGY: A cross-sectional study was conducted in 26 agricultural and 61 non-agricultural workers of both sexes (>18 years old) from Teresópolis city. Serum levels of 27 kinds of cytokines and chemokines, including FGF basic, IL-2, IL-10, MIP-1 α , Eotaxin, IL-4, IL-12, MIP-1 β , G-CSF, IL-5, IL-13, PDGF-bb, GM-CSF, IL-6, IL-15, RANTES, IFN- γ , IL-7, IL-17A, TNF- α , IL-1 β , IL-8, IP-10, VEGF, IL-1ra, IL-9, and MCP-1 were analyzed by multiplex immunoassay analysis. Pesticide use, sociodemographic and lifestyle information was collected through structured questionnaires. RESULTS: The analysis revealed that agricultural workers exhibited significantly lower levels of the IL-9, eotaxin, TNF α and MIP-1 β cytokines compared to non-agricultural workers ($p < 0.05$). No significant differences were observed for the other cytokines analyzed. CONCLUSION: These findings indicate an association between pesticide exposure and alterations in cytokine levels, supporting the need for larger studies on the immunotoxic effects of pesticides.

Keywords: Pesticide exposure;Cytokines;Rural population.

3476 - Category: CLINICAL IMMUNOLOGY (CL)

Longitudinal Monitoring of Plasma Biomarkers in Young Individuals with Vertically Transmitted HIV: Clinical Implications.

COELHO, M.P.¹; RIBEIRO, L.B.¹; TOQUETÃO, M.V.L.¹; THOMAZELLA, M.V.¹; SILVEIRA, C.G.T.¹; THOMÉ, B.D.C.²; PACOLA, D.P.³; KALLÁS, E.G.⁴. 1. FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. GLOBAL FUND TO FIGHT AIDS, TUBERCULOSIS AND MALARIA, GENEVA - SUICA; 3. INSTITUTO DE INFECTOLOGIA EMÍLIO RIBAS, SÃO PAULO - SP - BRASIL; 4. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

Chronic inflammation is common among people living with HIV (PLHIV) and is often associated with comorbidities. In those infected through vertical transmission (VT), the prolonged exposure to the virus and consequent higher basal immune stimulation can accelerate the onset of diseases commonly associated with aging. In this study, we evaluated age-associated inflammatory plasma markers at two timepoints (referred to as T1 and T2), seven years apart, in a cohort of PLHIV with VT infection and a median age of 21.5 (IQR: 17.5-25) in T2. Viral load (VL) was detectable in 4 out of 20 participants at T2. Cytokines and chemokines markers (such as IFN- γ , IL-1 β , IL-2, IL-18, IP-10, CRP and others) were analysed by electrochemiluminescence assays in plasma samples collected at T1 and T2. Plasma levels of CCL22 ($p=0.021$), sCD14 ($p=0.009$), and TNF- α ($p=0.002$) showed a decrease between T1 and T2 (Wilcoxon test). In the correlation analysis using Spearman test, negative correlations were found between age and TNF- α ($p=-0.68$; $p=0.001$) and CCL22 ($p=-0.52$; $p=0.01$). Recognizing the effect of age on the inflammatory profile in the context of VT, we observed a positive correlation between age and duration of antiretroviral therapy (ART), further reinforcing ART's influence on inflammatory responses. In addition, participants with detectable VL had higher levels of these markers, highlighting the importance of sustained viral suppression through adherence to ART. In conclusion, our data suggests that the joint decline in TNF- α , sCD14, and CCL22 levels reflects a possible reduction in systemic inflammatory activation in these individuals, which may be beneficial for the prevention of non-AIDS-related comorbidities, such as cardiovascular diseases, neuroinflammation and premature immunosenescence. Given the increased life expectancy among PLHIV, this study contributes to the development of targeted public health policies for this population.

Keywords: Vertical transmission of HIV; Inflammation; Immunosenescence.

3486 - Category: CLINICAL IMMUNOLOGY (CL)

Immunological Profile in Fibromyalgia: Comparative Analysis of Cytokines as Indicator of the Syndrome

SILVA, A.L.C.A.E.; LAMOGIA, A.S.A.; BRANDAO, S.G.; DESIDÉRIO, C.S.; BOVI, W.G.; MORAES, S.K.; DE OLIVEIRA, C.J.F.. UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO, UBERABA - MG - BRASIL.

Fibromyalgia (FM) is a high global prevalence idiopathic syndrome, characterized by complex and multifactorial symptoms such as widespread pain, fatigue, morning stiffness, depression and anxiety. Its diagnostic criteria have undergone several reformulations due to the absence of biomarkers, being currently established when pain is present in at least four of the five body regions and with its symptom severity assessed using different scales. Its treatment involves pharmacological interventions, including antidepressants, anticonvulsants, and muscle relaxants, as well as non-pharmacological approaches such as psychotherapy, physical therapy, regular exercise, and complementary therapies. The main limitation of FM still is the lack of specific biomarkers capable of confirming the diagnosis, as no test has demonstrated sufficient reliability to differentiate FM from other conditions. Therefore, this study aimed to analyze the immune profile of 102 FM patients treated at HC-UFTM, comparing them with 30 healthy control patients. Initially, seven cytokines were measured: TNF- α , IL-10, IL-6, IFN- γ , IL-1 β , IL-8, and IL-12. The results revealed that previously diagnosed FM patients exhibited higher cytokine concentrations compared to the control group, notably in the pro-inflammatory cytokines IFN- γ , IL-8, and IL-12, which play an important role in triggering inflammatory processes. These findings suggest a significant inflammatory component in the pathophysiology of FM, reinforcing the need for further investigations into the role of these cytokines as potential biomarkers or therapeutic targets.

Keywords: Fibromyalgia; Cytokines; Immunology.

3501 - Category: CLINICAL IMMUNOLOGY (CL)

OGG1 Ser326Cys polymorphism and nickel exposure in allergic contact dermatitis: A hospital-based Case-Control study

NOGUEIRA, S.M.; MOTA, C.D.L.; DA SILVA, C.H.; MAGALDI, K.M.L.; MUNIZ, M.F.R.. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Nickel (Ni) exposure has been associated with an increased Allergic Contact Dermatitis (ACD), a common inflammatory skin disease, that represents a type IV hypersensitivity reaction. Ni exposure occurs through a wide range of items. The rs1048943 variation in OGG1, a DNA repair gene, has been associated with increased oxidative DNA damage in ACD patients. **Objective:** This study aimed to investigate the potential association between the OGG1rs1048943 polymorphism and ACD, particularly cases triggered by Ni exposure, among patients with dermatoses. **Methodology:** A total of 246 patients from dermatology ambulatories participated in this Case-Control study. The diagnosis of ACD was established through clinical evaluation and epicutaneous testing. A Ni-specific skin test was used to identify ACD cases caused by Ni. Genotyping was performed using real-time PCR. Statistical analyses were conducted to compare variables across the groups, and evaluate possible associations between variables. **Results:** In the Case group, genotype frequencies were 61.7% for CC, 32.5% for CG, and 5.8% for GG, whereas the Control group showed frequencies of 63.7% for CC, 31.9% for CG, and 4.4% for GG. Both groups showed no deviation from Hardy-Weinberg equilibrium. A significant association was observed between Ni-induced allergic skin reactions and the OGG1 variant allele ($p=0.003$). Additionally, ACD prevalence was significantly associated with Ni ($p=0.004$, and also with chromium ($p<0.001$), and cobalt ($p=0.001$) allergens. **Conclusion:** The findings of this study suggest that the OGG1rs1048943 polymorphism was associated with cutaneous hypersensitivity to Ni, indicating a potential genetic susceptibility factor for ACD, specially in Ni-induced cases. Further gene-environment interaction studies are warranted to confirm these findings and support the development of Public Health policies and more effective strategies for the prevention and treatments of this prevalent allergic condition.

Keywords: Allergic Contact Dermatitis; Nickel exposure; DNA repair polymorphism.

3508 - Category: CLINICAL IMMUNOLOGY (CL)

Towards a Molecular Diagnostic: Immune and Microbial Signatures Differentiating Viral and Bacterial Tonsillitis

SILVA, C.A.C.¹; CASAIS, P.²; CEDRO, M.M.³; CARDOSO, C.R.D.B.⁴; TSCHOEKE, D.⁵; IEBBA, V.⁶; RAMOS, P.I.⁷; KHOURI, R.⁸; BOAVENTURA, V.⁸. 1. GONÇALO MONIZ INSTITUTE, OSWALDO CRUZ FOUNDATION (FIOCRUZ); ONCOCLINICAS&CO - MEDICA SCIENTIA INNOVATION RESEARCH (MEDSIR), SALVADOR - BA - BRASIL; 2. FEDERAL UNIVERSITY OF BAHIA (UFBA), SALVADOR - BA - BRASIL; 3. SANTA CASA DA BAHIA, SANTA IZABEL HOSPITAL, SALVADOR - BA - BRASIL; 4. UNIVERSITY OF SÃO PAULO (USP), RIBEIRÃO PRETO - SP - BRASIL; 5. DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND TRANSPLANTATION, REGA INSTITUTE FOR MEDICAL RESEARCH, KU LEUVEN (KATHOLIEKE UNIVERSITEIT LEUVEN), LEUVEN - BELGICA; 6. GUSTAVE ROUSSY CANCER CAMPUS, CLINICOBIOIME, VILLEJUIF - FRANÇA; 7. FIOCRUZ BAHIA, SALVADOR - BA - BRASIL; 8. FEDERAL UNIVERSITY OF BAHIA (UFBA); GONÇALO MONIZ INSTITUTE, OSWALDO CRUZ FOUNDATION (FIOCRUZ), SALVADOR - BA - BRASIL.

Acute tonsillitis, caused by viral or bacterial infections, poses a diagnostic challenge. Misclassification can lead to antibiotic overuse and resistance. Considering the local immune response may improve differential diagnosis. This study aimed to identify in situ immune response biomarkers distinguishing bacterial from viral tonsillitis. A cross-sectional study included 197 patients with suspected acute tonsillitis at Hospital Santa Izabel (Salvador, BA). Cases were classified as bacterial or viral based on the modified Centor Score and respiratory symptoms with tonsillar signs. Patients with recent antibiotic, anti-inflammatory, or immunosuppressive use were excluded. Swab samples were collected from palatine tonsils of patients and five healthy controls. mRNA expression was analyzed in 24 samples using NanoString nCounter, quantifying 770 immune-related genes. RNA (50 ng) was hybridized with a customized panel; assays were performed at KU Leuven. Differential expression analysis used $\log_2$ transformation, t-test, and 5% FDR correction. Functional enrichment (GSEA) was performed with gProfiler using GO, KEGG, Reactome, and WikiPathways databases. Twenty-four samples (10 bacterial, 9 viral, 5 controls) were analyzed. Bacterial tonsillitis showed upregulation of 29 genes, including *IL21R*, *CD47*, *ITGAM*, *CD163*, and downregulation of 11 genes such as *HLA-DRB5* and *TUBA3E* compared to viral cases ($p < 0.01$). Functional enrichment revealed distinct profiles: bacterial infections activated inflammation and antigen-presentation (MHC II) pathways; viral cases were linked to intracellular trafficking and cytoskeleton organization. These results highlight distinct transcriptomic signatures in bacterial and viral tonsillitis, suggesting the potential of in situ gene expression for improved diagnosis. However, validation in larger cohorts is needed.

Keywords: Microbiota; Transcriptomics; Tonsillitis.

2918 - Category: EDUCATION IN IMMUNOLOGY (EI)

Social Media and the Cult of the Body: Impact on Adolescent Use of Anabolic Steroids and Implications for Immune System Function

GOMES, A.C.F.; NASCIMENTO, I.A.; DOS SANTOS, R.Q.; SILVERIO, Y.A.D.C.; NASCIMENTO, D.D.O.; DECOTE-RICARDO, D.; DOS SANTOS, D.R.L.; DA SILVA, L.H.P.. UFRRJ, SEROPÉDICA - RJ - BRASIL.

The ImunoEnsinando-UFRRJ project aims to promote scientific information dissemination and the understanding of immunology concepts in schools in the Baixada Fluminense region, located near the Federal Rural University of Rio de Janeiro (UFRRJ). The project aims to explore emerging public health issues and their impact on the immune system among adolescents. In 2024, an interactive activity was held at a technical school with two third-year classes, involving approximately 36 students aged 16 to 20 years old. The objective was to explore how the misperception of idealized bodies, aggravated by social media, may lead to the use of anabolic steroids and how these substances have a negative impact on the immune system. The activity first explored topics such as how anabolic steroids affect immune system cells, compromising the body's homeostasis. Scientific articles were used to link the immunosuppression caused by anabolic steroids with the occurrence of diseases in their users. The activity concluded with a quiz using a game app to assess the concepts that had been learned. This quiz used questions related to the immune system, such as which immune cells are affected by these substances and how they are related to homeostasis, which cell is responsible for the body's first line of defense, and which cell produces antibodies. This quiz resulted in a 90% accuracy rate. Additionally, a questionnaire was conducted before and after the activity to assess the knowledge acquired. Results showed that, before the activity, 55.3% of the students were aware of the effects of steroid use, which increased to 97% after the activity. This highlights the importance of using interactive activities to discuss immunology concepts with the young public in schools.

Keywords: High School;Social media;Anabolic steroids.

2979 - Category: EDUCATION IN IMMUNOLOGY (EI)

Gamification in the teaching of immunology: Application of a living board game in an extension program

NAGIB, P.R.A.; SOBRINHO, M.C.F.; PEIXOTO, A.C.F.; BITTENCOURT, M.E.G.E.D.A.G.; CASTRO, J.M.D.A.. UFJF, JUIZ DE FORA - MG - BRASIL.

The concepts and actions of the immune system are complex and abstract, making them challenging to teach, especially to children. Children often have limited prior knowledge about biological process, and scientific language can make it difficult for them to understand. Game-based strategies can be pedagogical tools to promote active participation, and learning. In university extension, gamification combines scientific knowledge with interactive practices in a fun and accessible way. The aim was to develop and apply a board game designed to explore the relationship between nutrition and immunity, to be used in extension programs for children. The game was created based on a gamification approach with challenges, rewards, narrative elements, and decision-making. First, the educational aim was defined (the correlation between a healthy diet and immunity). Second, the type of game and how to play were chosen. Lastly, we created the narrative, questions, and answers. The game was conducted with 35 elementary school students (grades 3 and 4) from a private school in Juiz de Fora, MG, as part of a university extension project in Immunology for Nutrition. Using the living board game approach, the children were divided into groups and took on roles such as rolling the dice, moving around the board, and selecting food cards to address scenarios related to the immune system. The game's narrative involving the immune system and the impact of healthy eating, using to analogies, visual resources, and interactive activities. Themes such as immune cells, phagocytosis, antibodies, immunity, and vaccines were presented and related to the energy provided by a good and diverse diet. At the end, in assembly, children could orally express their understanding of immunity concepts, demonstrating that gamification can effectively enhance the teaching of complex topics in a fun and educational way. This new game provided significant learning opportunities and engagement for the participants.

Keywords: gamification;immune system;healthy eating.

3135 - Category: EDUCATION IN IMMUNOLOGY (EI)

CARTELAS: BOARD GAME FOR SCIENTIFIC OUTREACH AND EDUCATION ON CAR-T CELLS

BASTOS, M.R.¹; GULARTE, D.²; FEITOSA, D.³; MARANHÃO, A.Q.⁴; BRIGIDO, M.D.M.⁴; LOURENZONI, M.R.⁵; FURTADO, G.P.⁵; ALVES, P.D.S.¹; VIEIRA, L.M.¹; MARQUES, J.D.D.P.C.¹; DOS REIS, S.A.¹; SILVA, L.R.B.¹; DE LIMA, L.¹; STEIMBACH, J.V.¹; BONAMINO, M.H.¹; ALVES, G.⁴; PONCE, M.⁴. 1. INSTITUTO NACIONAL DE CÂNCER - INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UNICHRISTUS, FORTALEZA - CE - BRASIL; 3. ESTÚDIO DE JOGOS - GUARAH'S DEN, FORTALEZA - CE - BRASIL; 4. UNIVERSIDADE DE BRASÍLIA (UNB), BRASÍLIA - DF - BRASIL; 5. FUNDAÇÃO OSWALDO CRUZ CEARÁ (FIOCRUZ-CEARÁ), EUSÉBIO - CE - BRASIL.

Introduction: MartinLab in Schools is an outreach activity aimed at promoting science communication in the field of advanced therapies, such as CAR-T cell immunotherapy, for high school students. The project utilizes the educational board game CARTelas, as a tool to facilitate the understanding of key concepts including cancer, immune system, and CAR-T cell therapies. **Objective:** To promote the dissemination of knowledge in the field of advanced therapies with an emphasis on CAR-T cell immunotherapies among high school students through educational and interactive activities.

Methods: The educational activity was conducted with third-year high school students from *Colégio Estadual Souza Aguiar (CESA)*. Participants used the *CARTelas* game kit, which includes a printed board, instruction manual, tokens, and scientific question cards. The game is structured in three phases per round: scientific questions, maintenance of cell dynamics, and player actions for controlling or eliminating cancer cells. Victory is achieved by eliminating or containing tumor cells; uncontrolled proliferation results in defeat. **Results:** Third year high school students from CESA responded positively to the activity, demonstrating enthusiasm, especially when correctly answering the scientific questions. There were two rounds of the game, and the students defeated the tumor in both. **Conclusion:** The outreach activity was well received by students, who remained engaged throughout the session. The initiative successfully facilitated the understanding of key scientific concepts related to the immune system and CAR-T cell therapy in a interactive manner. Future improvements include refining the rulebook, redesigning cell pieces and questions, and adjusting session duration.

Keywords: Education;Immunology;CAR-T cells.

3399 - Category: EDUCATION IN IMMUNOLOGY (EI)

The Mediating Role of LAMIM Between Science and Society: Unveiling the Immune System

BORGES, A.B.M.¹; RIBEIRO, E.J.²; CARDOSO, M.F.²; DE LIMA, B.R.S.²; DE ARAUJO, T.L.²; LEONEL, G.M.²; DE OLIVEIRA, S.N.R.V.²; GOMES, D.L.R.²; FALCÃO, C.A.B.². 1. INSTITUTO FEDERAL DO RIO DE JANEIRO, NOVA IGUAÇU - RJ - BRASIL; 2. INSTITUTO FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

The field of immunology began in 1796, when Edward Jenner introduced the concept of vaccination by demonstrating that cowpox infection conferred protection against smallpox. Despite substantial scientific advancements, immunology remains poorly understood by the general public and is often perceived as distant from everyday life, largely due to its complex technical language. Aiming to bridge this gap, the Academic League of Microbiology and Immunology (LAMIM) at IFRJ/Campus Realengo aims to promote immunological knowledge in a more accessible and contextually relevant manner. Furthermore, misinformation among the general population contributes to unsafe practices, such as the unsupervised use of corticosteroids (Almeida et al., 2018). This project promotes scientific literacy and bridges theoretical concepts with clinical and real-life applications. Key activities include educational content on social media, public lectures, and short courses designed with comprehensible language, targeting diverse audiences. Noteworthy initiatives include the organization of the First LAMIM Week featuring hybrid lectures and hands-on workshops, and joint events with other institutions. The project's Instagram account reaches an average of 6,358 users every 30 days. In academic settings, the initiative engaged 25 students during the "Farmacologia Descomplicada" lecture at UERJ Summer Course and trained 21 participants in a syphilis diagnostic workshop. The activities received highly positive feedback, with participants reporting increased engagement, knowledge acquisition, and a shift in their perception of immunology. By addressing underexplored topics through accessible scientific communication, the project has effectively enhanced public understanding and emphasized the importance of scientific literacy and responsible health information dissemination. These outcomes underscore the relevance of maintaining and expanding the project's impact on society's critical.

Keywords: Scientific dissemination;Health education;Immune System.

3521 - Category: EDUCATION IN IMMUNOLOGY (EI)

WHEN IMMUNOLOGY MEETS ART: A NEW APPROACH TO TEACHING AND LEARNING IMMUNOLOGY

DA SILVA, A.C.B.; DOS SANTOS, D.C.M.; SIMÕES, A.C.B.L.; RUAS, T.C.B.; MACHADO, A.A.D.S.; TASCA, M.G.. IDOMED/UNIVERSIDADE ESTÁCIO DE SÁ, RIO DE JANEIRO - RJ - BRASIL.

Currently, several authors have been describing and increasingly valuing the inclusion of art in medical schools, which translates into a more humanized education and the development of important skills and competencies for medical practice. Art stimulates empathy, critical thinking, improves doctor-patient communication, and encourages active listening. Furthermore, art, as a learning tool, can lead to stress reduction and increased well-being. Our objective was to include art in immunology classes, aiming to reduce stress and improve learning in clinical case resolution. For the application of the methodology, we had the support of the Divercittà nucleus of our University, which has as one of its aspects art as a teaching resource and an institutional process of humanization. The methodology was applied to a group of thirty medical students, where the class topic was the immune response against viruses. The professors distributed the clinical cases. In the first stage, the students answered the questions from the clinical cases. In the second stage, the students responded to the clinical case with art. The art forms were chosen by the students themselves with the materials that were available in the classroom, so that the students could observe and create. In the third stage, the groups presented the response to the clinical case in the form of art. Our results were very rewarding. There was better interaction in the class, and all students reported a better understanding of the content covered. After the class, we collected some reports from the students about the new active methodology applied. We believe that the integration of art into the medical curriculum is important, which is increasingly recognized as fundamental for the development of important skills for training. When we associate art with immunology, we observe all these skills being built, in addition to the improvement of learning the presented content

Keywords: ART;IMMUNOLOGY;LEARNING.

2660 - Category: HUMORAL IMMUNOLOGY (HI)

Screening and evaluation of *Schistosoma mansoni* peptides as targets of serological tests for the schistosomiasis diagnosis

RESENDE, L.M.; ANDRADE, J.C.S.; FONSECA, C.T.; ANTONELLI, L.R.D.V.; MARTINS-FILHO, O.A.; PEREIRA, R.A.D.S.. INSTITUTO RENÉ RACHOU, BELO HORIZONTE - MG - BRASIL.

Despite the advances in schistosomiasis control, the disease remains a serious global public health issue. New diagnostic strategies are essential, as the currently used Kato-Katz parasitological method has low sensitivity. Serological tests offer a promising alternative for the diagnosis of *Schistosoma mansoni* infection, but the identification of specific antigens is necessary. Our group identified potential target proteins, including PPE, through immunoproteomics and bioinformatics. This project aims to select and evaluate antigenic peptides from *S. mansoni* proteins for new serological diagnostic tests. The experimental strategy was defined in two approaches: (1) *in silico* prospection of PPE-derived peptides, and (2) immunoassays using microarrays of peptide derived from PPE and other proteins. Six PPE peptides (P1–P6) were identified, synthesized, and P6 was tested by ELISA using serum from infected and non-infected individuals, previously characterized by Helmintex and Kato-Katz. The ELISA involved coating plates with P6 (6 µg/mL), blocking with 10% FBS, incubation with serum (1:80), anti-IgG-HRP (1:60,000), TMB substrate, and reading at 450 nm. The assay showed <5% variation but failed to distinguish *S. mansoni* infected from non-infected individuals. In parallel, custom peptide microarrays were synthesized, containing 726 15-mer peptides derived from five *S. mansoni* proteins: PPE, Smp33, Smp13, Smp18, and Smp19. Immunoassays were conducted using serum samples from infected and non-infected individuals, with fluorescently labeled antibodies. Two PPE-derived peptides (P7 and P8), strongly recognized in the microarray, were synthesized and tested by ELISA, but also failed to discriminate infection status. From the microarray, 120 peptides with the highest differential reactivity were selected for the construction of a new microarray, which will be used to assess their performance in a diagnostic test for schistosomiasis.

Keywords: Schistosomiasis;Serological Diagnosis;Peptide Microarray.

2724 - Category: HUMORAL IMMUNOLOGY (HI)

IMPLICATIONS OF HUMAN SPECIFIC N-GLYCOLYLNEURAMINIC ACID LOSS IN B CELL DYNAMICS AND INTESTINAL MICROBIOTA

CRUZ, P.C.O.D.M.¹; MAIA, L.C.R.¹; CAVAZZONI, C.B.¹; ZAMELA, L.S.²; ARIAS-CARRASCO, R.³; HIDALGO, A.R.⁴; COUTINHO, V.M.⁴; TODESCHINI, A.R.¹; NÓBREGA, A.¹; VALE, A.M.¹; ALISSON-SILVA, F.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL; 3. UNIVERSIDAD TECNOLÓGICA METROPOLITANA, SANTIAGO - CHILE; 4. UNIVERSIDAD DE CHILE, SANTIAGO - CHILE.

Every cell presents a dense layer of glycoconjugates on its surface, mostly terminated by two sialic acid isoforms: N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Humans lack Neu5Gc due to a mutation in the CMAH gene, which encodes the enzyme responsible for Neu5Gc synthesis, resulting in different gut microbiome profiles and immune cell dynamics. To investigate the influence of Neu5Gc loss on B cell compartments, antibody repertoire, and its potential correlation with intestinal microbiota, we performed flow cytometry of different B cell subsets in WT and CMAH^{-/-} mice, observing higher frequencies of B-1b and marginal zone B cells in the peritoneum and spleen of CMAH^{-/-} mice, respectively. Both populations comprise an immunoglobulin repertoire with both self- and pathogen-reactive clones. We also observed increased B cell numbers in the mesenteric lymph nodes of CMAH^{-/-} mice. B cell repertoire evaluation by blotting culture supernatants (SNs) against brain and muscle lysates revealed greater binding of IgM from CMAH^{-/-} SNs to both WT and CMAH^{-/-} lysates, suggesting that Neu5Gc absence influences IgM reactivity against self-antigens. However, CMAH^{-/-} mice presented lower immunoglobulin serum titers across all classes, indicating that B cell expansion does not correlate with serum antibody levels in this model. As gut microbiota is related with the natural antibody repertoire, we evaluated IgA-bound bacteria in fecal samples, revealing a higher number of IgA⁺ bacteria in WT mice, suggesting that Neu5Gc⁺ bacteria are more antigenic. Moreover, co-housing WT and CMAH^{-/-} mice led to increased IgM binding to muscle lysates and altered alpha- and beta-diversity in CMAH^{-/-} gut microbiota. In conclusion, the loss of Neu5Gc modifies different B cell compartments, enhances IgM reactivity and interferes with gut microbiota composition, highlighting the interplay between glycosylation, immune response and host-microbiota interactions.

Keywords: Neu5Gc; Natural antibodies; Microbiota.

2822 - Category: HUMORAL IMMUNOLOGY (HI)

Kinetics of maternal obesity effects on humoral immune response establishment in offspring spleen: a murine experimental model

DURAO, C.V.; IZOLAN, L.B.; ALVES, D.C.D.A.; SILVA, R.S.F.; DA SILVA, A.B.R.; DE SOUZA, V.P.; MENEGATI, L.M.; DE OLIVEIRA, E.E.; MANFRO, W.F.P.; SILVA, F.M.D.C.E.. UERJ, RIO DE JANEIRO - RJ - BRASIL.

The rising prevalence of maternal obesity has become a major concern due to its association with increased mortality and disease risks. During gestation, it may induces metabolic changes capable to affect fetal development, including immune system impacts. This study aimed to evaluate the effects of maternal obesity on the development of humoral immune response in offspring spleen. Female BALB/c mice were fed a high-fat diet (60% calories from fat) for ten weeks prior to mating. Offspring were analyzed at different ages (1, 7, and 24 days postnatal), with spleen and serum collected for cellular phenotyping by flow cytometry, histological analysis, and antibody isotype detection via ELISA. Flow cytometry revealed changes in cell population frequencies and membrane molecule expression in the spleen. On day 1, offspring from obese mothers showed reduced B lymphocyte populations and increased CD19⁺CD138⁺ plasma cells compared to controls. By day 7, we observed increased B lymphocytes and MHCII expression, alongside decreased CD19⁺CD138⁺IgG2a⁺ plasma cells and CXCR5⁺ T lymphocytes. At day 24, populations of B lymphocytes, plasma cells and T lymphocytes were increased, but with reduced MHCII and PD-L1 expression in CD19⁺ cells. Histological analysis demonstrated a increased number of lymphoid follicles and decreased eosinophil counts in offspring from obese mothers. Serum analysis revealed decreased IgM and total IgG levels, but increased IgG1 and IgG2a in later developmental stages. These results indicate that maternal obesity impairs humoral immune response development. Reduced T lymphocytes and low IgGTotal/IgM levels at 7th day suggest compromised class switching and immunological memory, potentially leading to less efficient immune responses and prolonged infections. This may contribute to immune dysfunction at later stages, as observed at 24th day. I gratefully acknowledge the funding agencies essential for the development of this research: CAPES, CNPq, and FAPERJ.

Keywords: Maternal obesity; Humoral immune response; Murine experimental model.

2941 - Category: HUMORAL IMMUNOLOGY (HI)

Early humoral response with high affinity antibodies is a predictor of viral clearance in SARS-CoV-2 infection in non-persistent patients

RODRIGUES, D.A.S.¹; GABRIELLE, B.¹; MAIA, L.C.R.¹; DE FREITAS, L.R.C.¹; PESTANA, J.D.C.¹; NERY, B.¹; BOZZA, V.B.T.¹; RAPOSO, L.M.²; GAMA, A.M.D.S.¹; VIDAL, V.M.¹; COBOS, E.V.M.¹; DE LIMA, J.E.N.¹; GALLIEZ, R.¹; CASTIÑEIRAS, T.M.P.P.¹; CASTILHO, L.¹; TANURI, A.¹; FERREIRA JR, O.C.¹; BOZZA, M.T.¹; VALE, A.M.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIRIO, RIO DE JANEIRO - RJ - BRASIL.

The health crisis caused by COVID-19 has highlighted the complexity of immune responses, which vary significantly between individuals. One of the remaining gaps in understanding SARS-CoV-2 immunity is how early humoral responses, particularly the timing and quality of antibody (Ab) production, correlate with viral clearance. Although this study was conducted after the peak of the pandemic, immunological data on the Brazilian population remain limited, especially regarding early Ab conversion and the persistence of viral RNA. Understanding these factors is fundamental to developing effective disease control strategies. In this study, we investigated differences in Ab responses and their correlation with viral clearance, seroconversion, and vaccine-induced protection, aiming to define immunological profiles associated with distinct stages of infection and responses to the ancestral SARS-CoV-2. We analyzed the longitudinal humoral immune response of healthcare workers who contracted SARS-CoV-2 and presented with mild to moderate symptoms, classifying them according to viral persistence days after symptom onset (DASO) as "non-persistent" (viral clearance < 24 DASO) and "persistent" (> 25 DASO). Blood plasma analyses revealed that non-persistent individuals developed an early (0-11 DASO) and robust specific humoral response (IgM, IgA, IgG anti-Spike/RBD), with higher seroconversion, high-affinity Ab titers, and viral load control by IgM. Persistent individuals had slower responses (after 12-17 DASO for S/RBD). In both groups, IgG1 predominated. The lack of correlation between global IgM/IgG and specific anti-SARS-CoV-2 Abs suggests that the assessment of protective immunity should focus on specific Abs. After infection, both groups maintained high and long-lasting levels of IgG anti-S/RBD, sustained by vaccination. Vaccination induced an increase in IgG2 and, notably, IgG4 anti-S, indicating maturation of the response and being essential for maintaining affinity Ab titers against the S protein.

Keywords: SARS-CoV-2; Antibodies; Humoral response.

2968 - Category: HUMORAL IMMUNOLOGY (HI)

BINDING OF ANTI-TOLC ANTIBODIES IN BACTERIA OVEREXPRESSING EFFLUX PUMPS DUE TO PRESSURE INDUCED BY ANTIMICROBIALS

DOS SANTOS, Y.B.; TEIXEIRA, B.D.A.; OLSEN, P.C.; DA COSTA, T.L.; MOREIRA, L.O.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Resistance to antimicrobials is a serious public health concern. Among the resistance strategies of Gram-negative bacteria, efflux pumps stand out for reducing intracellular drug concentration and limiting treatment efficacy. Overexpression of such proteins is associated with resistance, making them potential therapeutic targets. TolC is a transmembrane protein forming the outer membrane component of several efflux pumps. Our group has shown that TolC is immunogenic and antibodies anti-TolC display a protective effect when immunized mice were infected. This study aimed to induce antimicrobial resistance by adapting bacteria to increasing antibiotic concentrations and to evaluate anti-TolC antibody binding in bacteria overexpressing efflux pumps. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700063 were used. Bacteria were exposed to increasing chloramphenicol concentrations to promote selective pressure and induce efflux pump overexpression. The resistance induction protocol was based on a previously determined MIC of 1.9 µg/ml for both strains. MacConkey agar plates were prepared with various antibiotic concentrations: no antibiotic, 1/2x MIC, 1x MIC, 2x MIC, 4x MIC, 8x MIC, and 16x MIC. Bacteria were first seeded on plates without antibiotics, then transferred sequentially to higher concentrations. During this process, protein and RNA extractions were performed for Western blotting (WB). Mouse immune serum containing anti-TolC antibodies was used for detection of TolC by WB. Preliminary results show increased TolC expression in bacteria grown at 4x MIC. Resistance phenotype was confirmed by increased MIC values. Immune serum anti-TolC presented increased antibody binding in bacteria with increased antimicrobial resistance. These findings are crucial to understanding resistance mechanisms and advancing alternative therapeutic approaches.

Keywords: Antimicrobial resistance; Efflux pumps; Antibodies.

2977 - Category: HUMORAL IMMUNOLOGY (HI)

Antibody recognition of SARS-CoV-2 variants following primary Wuhan infection: impact of viral persistence and vaccination

PESTANA, J.D.C.¹; GABRIELLE, B.¹; MAIA, L.C.R.¹; DE FREITAS, L.R.C.¹; NERY, B.¹; BOZZA, V.B.T.¹; RAPOSO, L.M.²; GAMA, A.M.D.S.³; VIDAL, V.M.³; COBOS, E.V.M.³; DE LIMA, J.E.N.³; GALLIEZ, R.⁴; CASTIÑEIRAS, T.M.P.P.⁵; CASTILHO, L.⁶; TANURI, A.⁷; JÚNIOR, O.D.C.F.⁸; BOZZA, M.T.³; VALE, A.M.¹; RODRIGUES, D.A.S.¹. 1. INSTITUTE OF BIOPHYSICS CARLOS CHAGAS FILHO, PROGRAM IN IMMUNOBIOLOGY, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FEDERAL UNIVERSITY OF THE STATE OF RIO DE JANEIRO, CENTER FOR EXACT SCIENCES AND TECHNOLOGY, RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTE OF MICROBIOLOGY, IMMUNOLOGY DEPT., UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 4. MEDICAL SCHOOL, INFECTIOUS AND PARASITIC DISEASE DEPT., RIO DE JANEIRO - RJ - BRASIL; 5. MEDICAL SCHOOL, INFECTIOUS AND PARASITIC DISEASE DEPT., UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 6. COPPE, CHEMICAL ENGINEERING PROGRAM. CELL CULTURE ENGINEERING LAB, RIO DE JANEIRO - RJ - BRASIL; 7. BIOLOGY INSTITUTE, GENETICS DEPT., MOLECULAR VIROLOGY LAB., RIO DE JANEIRO - RJ - BRASIL; 8. BIOLOGY INSTITUTE, GENETICS DEPT., MOLECULAR VIROLOGY LAB., UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Since the onset of the COVID-19 pandemic, SARS-CoV-2 has accumulated successive mutations, giving rise to variants with distinct profiles of transmissibility, immune evasion, and clinical severity. The antibody (Ab)-mediated immune response plays a critical role in protection. However, mutations in the Spike protein, the main target of neutralizing Abs, can compromise immune responses elicited by prior infections or vaccination. Understanding the capacity of B cells to recognize and produce Abs against SARS-CoV-2 variants, as well as the kinetics serum Ab responses, is essential to reduce the risk of severe disease. In this study, we analyzed the capacity of antibodies induced by primary infection with the ancestral Wuhan strain to recognize variants of concern (Beta, Delta, Gamma, and Omicron) and whether prior infection with seasonal coronaviruses would alter the course of the COVID-19 response. The cohort was stratified according to viral persistence in the early infection phase into persistent individuals (viral RNA detectable >25 days after symptom onset, DASO), non-persistent individuals (<24 DASO), and uninfected controls. Peripheral blood and nasopharyngeal samples collected between March 2020 and February 2023 were analyzed by ELISA for anti-S IgG and by qRT-PCR for viral load. Our findings indicate that primary infection with the ancestral strain induced low cross-reactivity, with reduced levels of anti-Spike IgG against variants of concern during the acute phase. Notably, viral persistence was associated with higher levels of cross-reactive Abs, particularly against Gamma and Omicron, suggesting that prolonged antigen exposure may broaden humoral immunity. Omicron exhibited the highest level of immune escape. Vaccination, both primary and booster dose, was essential for enhancing and sustaining anti-S IgG responses across all variants in previously infected individuals. Despite the existence of Abs against seasonal coronaviruses, these did not influence the course of the infection. Keywords: Cross-reactive; SARS-CoV-2; Antibodies.

3002 - Category: HUMORAL IMMUNOLOGY (HI)

IgE Avidity After COVID-19 Vaccination: Insights From a Healthcare Brazilian Cohort

OLIVEIRA, G.S.¹; PORTILHO, A.I.¹; SILVA, V.O.²; DA COSTA, H.H.M.³; YAMASHIRO, R.⁴; DE OLIVEIRA, I.P.⁵; DE CAMPOS, I.B.⁶; PRUDENCIO, C.R.³; MATSUDA, E.M.⁵; BRÍGIDO, L.F.D.M.²; DE GASPARI, E.³. 1. UNIVERSITY OF SÃO PAULO/ ADOLFO LUTZ INSTITUTE, SÃO PAULO - SP - BRASIL; 2. DISEASE CONTROL COORDINATION/ADOLFO LUTZ INSTITUTE, SÃO PAULO - SP - BRASIL; 3. UNIVERSITY OF SÃO PAULO/ADOLFO LUTZ INSTITUTE, SÃO PAULO - SP - BRASIL; 4. ADOLFO LUTZ INSTITUTE, SÃO PAULO - SP - BRASIL; 5. SANTO ANDRÉ HEALTH SECRETARY, SANTO ANDRÉ - SP - BRASIL; 6. ADOLFO LUTZ INSTITUTE, SANTO ANDRÉ - SP - BRASIL.

Immunoglobulin E (IgE) antibodies, traditionally linked to allergies and parasites, are also increasingly recognized for binding viral antigens like SARS-CoV-2. A study investigated IgE response to SARS-CoV-2 Receptor Binding Domain (RBD) in a cohort of 59 Brazilian healthcare workers from the Adolfo Lutz Institute. The research utilized ELISA and avidity-ELISA techniques, with IgG depletion for confirmation. Sera were collected pre-vaccination (with or without COVID-19 history), after two CoronaVac or ChAdOx1 doses, and post-BNT162b2 booster. Pre-pandemic sera (30 samples) served as controls to establish the assay cutoff and calculate the ELISA index (IE = D.O. sample/cutoff). The study had ethical approval (CAAE 31924420.8.0000.0059). Natural COVID-19 infection led to a mild IgE increase (median; IQR25-75: 0.79; 0.64-1.46) with intermediate avidity (51.19; 35.95-57.78). After two vaccine doses, it was observed a slightly enhance in IgE levels (2; 1.35-2.95) and avidity (55.74; 23.92-76.25). Following BNT162b2 booster, IgE levels were even more increased (3.62; 3.27-4.52) ($p < 0.001$ compared to control), as well as avidity (80.79; 76.52—91.18). No statistical difference in IgE levels or avidity was found between CoronaVac+BNT162b2 and ChAdOx1+BNT162b2 regimens. IgE levels and avidity correlated well with neutralizing antibodies ($r = 0.7125$ and $r = 0.7967$, respectively) after two doses of CoronaVac or ChAdOx1. IgG depletion confirmed IgE detection specificity. IgG4 was detected after booster ($p < 0.001$ compared to control) and correlated moderately with IgE ($r = 0.3408$), with no regimen differences. No symptoms or comorbidities were linked to IgE response in the cohort. Further functional characterization is necessary to understand IgE's biological role in SARS-CoV-2 immunity. Keywords: IgE; SARS-CoV-2; Vaccines.

3068 - Category: HUMORAL IMMUNOLOGY (HI)

CNS autoreactivity in mothers and children with Congenital ZIKV infection

CAVALCANTE, S.D.F.¹; JERÔNIMO, S.M.B.²; MENDES-AGUIAR, C.D.O.²; NÓBEREGA, A.¹; BELLIO, M.¹; VALE, A.M.¹; SEQUERRA, E.B.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE, NATAL - RN - BRASIL.

The Congenital Zika Virus Syndrome (CZS) represents severe fetal neurological outcomes. While direct viral effects on the fetal central nervous system (CNS) have been partially elucidated, the maternal immune contribution to phenotypic variability of brain malformations remains poorly understood. Here, we investigate whether ZIKV infection during pregnancy promotes the production of maternal autoantibodies targeting CNS proteins and fetal neural antigens.

Serum samples from 28 pairs of mothers and children affected during the 2015-16 ZIKV outbreak in Brazil (Natal- RN) were analyzed. Western blot assays were performed using human brain and astrocyte protein extracts. Sandwich ELISAs were conducted for the detection of anti-Engrailed-1 (a homeobox protein highly expressed in the developing brain) antibodies, and using a kit to detect autoantibodies produced in basal ganglia-associated encephalitis.

Our preliminary data revealed a greater number of immunoreactive bands in the western blots from infected dyads compared to uninfected controls (mothers with no infection history during pregnancy). ELISA results showed significantly elevated anti-Engrailed-1 antibody levels in infected mothers but not in CZS children. The ELISAs for basal ganglia autoreactivity revealed a high expression of antibodies in both mothers and children with CZS. When the data for ELISA were normalized for total IgG expression, though, both mother and children had a high expression of anti-Engrailed-1, while basal ganglia autoantibodies were not differentially expressed than controls.

Our findings indicate that ZIKV infection during gestation may disrupt maternal-fetal immune tolerance, leading to a specific humoral response against fetal neurodevelopmental antigens. The selective increase in anti-Engrailed-1 antibodies supports a targeted, rather than generalized, autoreactive profile potentially contributing to CZS pathogenesis.

Keywords: autoreactivity; pregnancy; Congenital ZIKA virus syndrome.

3073 - Category: HUMORAL IMMUNOLOGY (HI)

Development and Optimization of a Human Monoclonal Antibody Against Zika Virus in the Offspring of Susceptible Pregnant Mice

AZEVEDO, I.R.¹; SILVA, G.A.D.S.¹; ANTONIO, N.D.S.¹; OTÁLORA, N.A.¹; ABREU, K.E.D.S.¹; SPECKHART, D.¹; ALMEIDA, B.D.S.²; YAMAMOTO, M.M.¹; BOSCARDIN, S.B.¹. 1. INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. IMUNOTERA SOLUÇÕES TERAPÊUTICAS E INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Zika fever is caused by the Zika virus (ZIKV), a flavivirus from the *Flaviviridae* family. Infection is typically mild, with symptoms like fever, conjunctivitis, and rash. However, between 2013 and 2014, ZIKV was linked to neurological complications such as Guillain-Barré syndrome. In 2015, following the virus's introduction into the Americas, Brazil reported a surge in microcephaly cases, later associated with intrauterine ZIKV infection. This condition, known as Congenital Zika Syndrome (CZS), involves a range of structural and functional abnormalities in the fetal central nervous system. In 2016, the World Health Organization declared the Zika epidemic a Public Health Emergency of International Concern.

Currently, there is no specific treatment or approved vaccine for ZIKV. However, highly neutralizing antibodies have emerged as a promising approach to prevent or treat infection. After the 2015–2016 outbreak, several groups began developing human monoclonal antibodies (mAbs) targeting ZIKV. Our research team produced a human anti-ZIKV mAb, A9Z, which demonstrated strong inhibitory activity in vitro and in vivo. To improve its therapeutic potential, we introduced two mutations in its Fc region: (1) LALA, to reduce Fcγ receptor binding and lower the risk of antibody-dependent enhancement (ADE); and (2) LS, to enhance pH-dependent binding to the neonatal Fc receptor (FcRn), thereby extending serum half-life. These modified mAbs will undergo in vitro characterization and in vivo testing at multiple post-infection time points. The most effective variants will then be administered to pregnant AG129 mice to evaluate their potential against CZS. **Funding:** Supported by São Paulo Research Foundation (FAPESP, grants #2024/04426-7 and #2024/01667-3), the Brazilian National Research Council (CNPq, grant #307596/2023-0), and Coordination for the Improvement of Higher Level Personnel (CAPES, finance code 001).

Keywords: ZIKV; Congenital Zika Syndrome; Monoclonal Antibody.

3149 - Category: HUMORAL IMMUNOLOGY (HI)

Murine antibody 4B1D3 exhibits broad cross-reactivity and neutralizing activity against SARS-CoV-2 variants of concern

DAHER, I.P.¹; SILVA, M.D.O.²; IBRAHIM, C.H.O.²; DE SOUZA, E.E.²; POSTOL, E.¹; DE ALENCAR, R.E.¹; SILVA, G.A.D.S.²; MARQUES, R.F.²; ADAMI, F.L.¹; SCHUCH, V.³; YAMAMOTO, M.M.²; ALMEIDA, B.D.S.²; KOIKE, G.²; AZEVEDO, I.R.²; AMARANTE, M.F.²; ROSA, D.S.⁴; DURIGON, E.²; WRENGER, C.²; CUNHA-NETO, E.¹; KALIL, J.¹; BOSCARDIN, S.B.². 1. FACULDADE DE MEDICINA, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. INSTITUTO CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. MOREHOUSE SCHOOL OF MEDICINE, ATLANTA - ESTADOS UNIDOS DA AMERICA; 4. DEPARTAMENTO DE MICROBIOLOGIA, IMUNOLOGIA E PARASITOLOGIA, UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Background: The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), identified in late 2019, led to a global COVID-19 pandemic, prompting an unprecedented international response in vaccination and public health strategies. Although the pandemic is now under control, the global spread of variants of concern (VOCs) has resulted in reduced efficacy of vaccines and monoclonal antibodies (mAbs) therapies, highlighting the urgent need for broad-spectrum therapeutic and preventive solutions. **Aim:** To generate murine mAbs using hybridoma technology and evaluate their binding and neutralizing activity against the SARS-CoV-2 VOCs. **Methods:** C57BL/6 mice (n=3) were intraperitoneally immunized with three doses of 10 µg Wuhan Spike protein with 50 µg of Poly I:C adjuvant, followed by a 10 µg Spike booster on day 33. Splenocytes were harvested and fused with myeloma cells to generate hybridomas. **Results:** Four mAbs were selected and purified by protein G affinity columns. Their structural integrity was confirmed by a 12% SDS-PAGE gel electrophoresis. Subclass analysis showed that 2B9F9 and 3E5G8 mAbs belong to the IgG2b subclass, while 4B1D3 and 4H4A2 mAbs to IgG2c. All mAbs recognized the SARS-CoV-2 Wuhan strain in infected cells by immunofluorescence assay. However, binding assays against the receptor binding domain (RBD) of the VOCs showed differential reactivity and loss of binding with the VOCs. Notably, the 4B1D3 mAb exhibited the broad binding and pseudovirus neutralizing activity across all tested variants, including Omicron BA.2, BA.4/5, and XBB.1.5. Furthermore, a single prophylactic dose of the 4B1D3 mAb provided protection in K18-hACE2 mice against a lethal challenge with SARS-CoV-2 Wuhan strain. **Conclusion:** The 4B1D3 mAb represents a promising tool for research and diagnostics, with significant potential for the development of new therapeutic strategies against SARS-CoV-2 variants. **Keywords:** Monoclonal antibody; SARS-CoV-2 variants of concern; neutralization.

3201 - Category: HUMORAL IMMUNOLOGY (HI)

The Kambô ritual and Immune System Activation: A Study on the Identification of Specific Antibodies to *Phyllomedusa bicolor* secretions

PICCOO, R.L.; VALENTIM, C.; FILARDI, E.T.M.; FERNANDES, M.R.; PUCCA, M.B.. UNESP, ARARAQUARA - SP - BRASIL.

Kambo is a waxy secretion extracted from the Amazonian giant monkey frog, *Phyllomedusa bicolor*, traditionally used by various Indigenous tribes of the Amazon in therapeutic rituals aimed at treating infections and preventing diseases. In addition to its analgesic properties, Kambo is known to induce profound physical and mental revitalization and is commonly associated with strengthening the body's natural defenses (i.e., immune system). In recent decades, the practice has gained popularity in urban centers worldwide, often marketed as a form of "natural vaccine". However, unsupervised use of Kambo has led to serious adverse reactions, including hospitalizations and, in some cases, fatalities. The secretion of *P. bicolor* produce acute pharmacological effects, with nausea, vomiting, tachycardia, and hypotension being the most common and characteristic reactions during the ritual. From an immunological perspective, the secretion contains peptides with antimicrobial properties that contribute to the frog's innate immunity. In humans, Kambo exposure can trigger an acute inflammatory response, potentially activating neutrophils. However, evidence of an adaptive humoral immune response, specifically the production of antibodies against *P. bicolor* components, remains scarce in the scientific literature. This study investigates the presence and magnitude of specific antibodies in individuals who underwent the Kambo ritual, aiming to clarify whether exposure induces a measurable humoral immune response. Serum samples from participants and matched controls were analyzed using indirect ELISA and biolayer interferometry (BLI, Octet system) to detect and quantify antibodies against the secretion's peptide components. By addressing a critical gap in current knowledge, this work provides the first systematic evaluation of the human adaptive immune response to Kambo and establishes a foundation for future studies exploring its immunological consequences including potential biomarkers.

Keywords: Kambo; *Phyllomedusa bicolor*; Antibody.

3277 - Category: HUMORAL IMMUNOLOGY (HI)

Chikungunya Pseudovirus Neutralization Assay for the Analysis of Protective Humoral Immunity.

ESCOTE-FERRAZ, A.C.; PORTES JUNIOR, J.A.; CALABRIA, P.A.L.; GIMENES, S.N.C.; MAGALHÃES, G.S.; MAURO, E.F.D.L.. BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL.

The Chikungunya virus (CHIKV) is an arbovirus from the *Togaviridae* family and *Alphavirus* genus, its transmission is primarily through mosquito bites of *Aedes aegypti* or *Aedes albopictus* species. The CHIKV symptoms vary from fever, intense articular pain, skin rash, nausea and vomiting to encephalitis and polyarthralgia. Since 2015 the number of CHIKV infections have been increasing in Brazil and, to date, there is no licensed vaccine or specific antiviral treatment. The antibodies are essential against viruses, exerting a relevant role blocking viral attachment to the cell and then decreasing the infection/replication, and increasing opsonization and phagocytosis. Thus, the detection of virus-specific neutralizing antibodies (NABs) in vaccinated or infected individuals indicates a protective and long lasting immunity. In this sense, our aim is to establish an *in vitro* assay to measure NABs in sera from infected and vaccinated individuals using a CHIKV pseudovirus-based cellular infection platform. The hCHIKV plasmid was amplified in the bacterial system. HEK293T17 cell culture was used to produce the CHIKV pseudovirus by transfection of hCHIKV, pAX2 and pHAGE plasmids. The infectivity of the obtained pseudovirus was evaluated in VERO E6, HeLa and A549++ cell lines via Luminometer and FluoroSpot equipment. The preliminary results showed that the CHIKV pseudovirus was able to infect all the cell lines, with higher infection in VERO E6 and A549++ cells (Statistical analysis by linear regression and 2-way ANOVA/Bonferroni). Then, the title of CHIKV was determined in the VERO E6 cells. The next steps include the confirmation of these results and the optimization of this assay using CHIKV pseudovirus aiming to evaluate the capacity of sera samples from infected individuals to neutralize the infection of cells by CHIKV pseudovirus.

Supported by CAPES and CeVIVAS (FAPESP- 2021/11944-6).

Keywords: Chikungunya;Pseudovirus;Neutralizing Antibodies.

3320 - Category: HUMORAL IMMUNOLOGY (HI)

Adaptive Thermogenesis immunity: the Role of B Cells in Thermogenic Adipose Tissues

DA SILVA, A.B.C.; DE MELO, P.; LEIRIA, L.O.S.. USP, RIBEIRÃO PRETO - SP - BRASIL.

Adaptive thermogenesis is the process of non-shivering heat production essential for maintaining energy homeostasis during cold exposure. This mechanism is primarily mediated through activation of brown adipose tissue (BAT) and the browning of white adipose tissue (iWAT). Thermogenic adipose tissues harbor diverse immune cell populations, which have emerged as key regulators of adipose tissue metabolism. Here, we demonstrate that B cell populations in both BAT and iWAT significantly expand following 7 days of cold exposure, yet their functional contribution to thermogenic capacity remains to be explored. Our previous findings revealed that B cell-deficient mice ($\mu\text{MT}^{-/-}$) born to $\mu\text{MT}^{-/-}$ dams exhibit impaired cold tolerance despite elevated energy expenditure and enhanced BAT metabolic activity. Notably, $\mu\text{MT}^{-/-}$ offspring from heterozygous crosses displayed comparable energy expenditure, glucose homeostasis, and cold tolerance relative to WT littermates, indicating a critical maternal factor in this phenotype. To directly assess B cell function in thermogenesis, we employed CD19^{cre}DT^R^{flx/flx} mice and observed that acute B cell depletion via diphtheria toxin administration paradoxically improved cold tolerance, suggesting a regulatory role for B cells in adaptive thermogenesis. To delineate the contribution of humoral immunity, we utilized IgMi mice (maintain normal B cells but lack secreting antibodies). Similar to $\mu\text{MT}^{-/-}$ mice, IgMi mice show reduced body temperature at 22°C and during cold challenge. Conversely, MHC class II-deficient mice, which lack T cell-dependent B cell activation and exhibit significantly reduced serum IgG, IgG1, and IgG2c levels, maintained normal acute and chronic adaptive thermogenesis comparable to control mice without alterations in food intake or glucose homeostasis. Collectively, these findings establish a critical role for B cell-derived antibodies in maintaining thermogenic adipose tissue function through T cell-independent mechanisms.

Keywords: B cells;thermogenesis;immunometabolism.

3431 - Category: HUMORAL IMMUNOLOGY (HI)

In Silico Studies of Diagnostic Targets for Lyme Disease: Challenges and Opportunities for Broad-Spectrum Testing

ATAIDES, L.S.; DE LEMOS, E.R.S.; RODRIGUES-DA-SILVA, R.N.. INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL

INTRODUCTION: The *Borrelia spp.* are the causative agents of Lyme disease (LD), the most prevalent tick-borne disease worldwide, and relapsing fever (RF). Serological methods are the foundation of LD diagnosis; however, their cross-reactivity with other pathogens may compromise specificity, especially in regions where relapsing fever and other spirochetes co-circulate.

OBJECTIVES: To evaluate the conservation of key proteins used in LD serodiagnosis and their B-cell epitopes across *Borrelia spp.* causing LD and RF, as well as potential cross-reactivity with other pathogens.

METHODS: A combination of nine immunoinformatic algorithms was utilized to predict linear B-cell epitopes of OspC, BmpA, Flagellin, VlsE, DbpA, and OspA. Linear B-cell epitopes ≥ 8 amino acids, predicted as antigenic by at least five algorithms, were selected for further analysis. The identities of proteins and their epitopes were compared among species related to LD and RF, and compared with epitopes from other pathogens to assess potential cross-reactivity.

RESULTS: Thirty B-cell epitopes were identified, of which 25 had prior experimental validation in *B. burgdorferi*. OspA showed high conservation among LD-related species, supporting its diagnostic potential. Conversely, Flagellin displayed extensive similarity with epitopes from RF-related *Borrelia* and other spirochetes, indicating a significant risk of cross-reactivity in co-endemic areas. Additionally, 16 epitopes exhibited similarity to human or non-borrelial antigens, suggesting possible interference in patients with autoimmune or concurrent infectious diseases.

CONCLUSION: These findings highlight the inherent cross-reactivity risks in current LD serodiagnostic targets and underscore the importance of careful clinical and epidemiological evaluation prior to testing. Insights from this study may guide the refinement of more specific and broad-spectrum diagnostic assays for Lyme disease.

Keywords: Lyme disease;Immunodiagnosis;Immunoinformatics.

3478 - Category: HUMORAL IMMUNOLOGY (HI)

Humoral and Cellular Responses to TolC Immunization: Effects on Microbiota and Maternal-Fetal Immunity

DA SILVA, G.M.; DE OLIVEIRA, A.C.D.S.C.; OLSEN, P.C.; VIDAL, V.M.; RIBEIRO, W.; FRAGA JUNIOR, V.D.S.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

The adaptive immune system exhibits a remarkable ability to generate highly specific receptors, a mechanism essential for defense against infections, including those caused by commensal microorganisms such as *Escherichia coli* (*E. coli*), which may behave as pathobionts. A virulence mechanism in this bacterium involves overexpressing the AcrAB-TolC efflux pump; the TolC subunit, embedded in the outer membrane, is immunogenic and displays epitopes recognizable by antibodies. Although two doses of recombinant TolC confer protection against multidrug-resistant *E. coli*, its effects on intestinal mucosal immunity and microbiota remain unclear. This study evaluates humoral and cellular responses in intestinal mucosa (colonic lamina propria, mesenteric lymph node, and Peyer's patches) and systemically, following intraperitoneal immunization of C57BL/6 mice with recombinant TolC+Alum. Non-immunized mice showed stable natural anti-TolC IgM production in the peritoneal lavage across age groups, with significantly higher levels in adult mice spleens. Anti-TolC IgG levels increased in neonate lavage ($p < 0.0001$), and IgA in 4- and 8-week-old groups. Seven days after a single dose of TolC + Alum, IgG⁺ memory B cells (MBC) increased in the mesenteric lymph node, with reductions in B220⁺ cells and IgM⁺ plasmablasts (Pb). In Peyer's patches, IgG⁺/IgA⁺ MBC and CD8⁺ T cells increased, while CD4⁺ T cells decreased. In the lamina propria, increases occurred in total B cells, IgG⁺/IgA⁺ MBC, IgM⁺/IgG⁺ Pb, IgG⁺/IgA⁺ plasma cells (B220-Blimp1⁺), and CD3⁺, CD4⁺, and CD8⁺ T cells. After three doses (42dpi), a sustained rise in serum anti-TolC IgG was detected, with no changes in total or specific IgM and IgA levels. In the intestine, CD4⁺ and Th17 increased in Peyer's patches, while lamina propria showed reductions in CD45⁺, CD4⁺, Th17, and IgM⁺ Pb. TolC proved highly immunogenic and capable of modulating mucosal and systemic immunity, with a long-term return to homeostasis.

Keywords: TolC immunization;Mucosal immunity;Microbiota.

2630 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Performance Evaluation of the Chagas-Flow ATE Serological Method by Flow Cytometry for Post-Treatment Monitoring of Chagas Disease

DOS SANTOS, L.M.P.¹; ALESSIO, G.D.¹; FIGUEIREDO, A.C.C.¹; SANTOS, S.M.E.²; RAMÍREZ, J.D.³; DE LANA, M.⁴; CARVALHO, A.T.¹; MARTINS-FILHO, O.A.¹. 1. FIOCRUZ-MINAS, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDAD DEL ROSARIO, BOGOTÁ - COLOMBIA; 4. UNIVERSIDADE FEDERAL DE OURO PRETO (UFOP), OURO PRETO - MG - BRASIL.

The therapeutic efficacy of Chagas disease (CD) can be impacted by the "Discrete Typing Units" (DTUs) in the infection of *T. cruzi*. However, the laboratory methods available for follow-up monitoring of chronic phase CD represent a challenge during clinical management, making it necessary to develop new methods for follow-up monitoring of chronic CD patients. In this context, the serological method for the detection of IgG1 anti-forms amastigote (A), trypomastigote (T) and epimastigote (E) of *T. cruzi* by flow cytometry (Chagas-Flow ATE) was standardized for the universal and genotype-specific diagnosis of CD. The objective of this study was to evaluate the performance of Chagas-Flow ATE in the follow-up monitoring of chronic CD. The reactivity of 77 serum samples from chronic CD untreated (NT), treated with Benznidazole at 5 years follow-up (BZ1), 15-25 years follow-up (BZ2) and 26-45 years follow-up (BZ3) patients were evaluated by this method. The "A", "T" and "E" forms of *T. cruzi* from the DTUs TcI, TcVI and TcII were cultured and prepared to be used as antigen in this method. Then, Chagas-Flow ATE serology and reading on the flow cytometer were performed. The results showed that when tested with "T" and "E" antigens of TcI, at a dilution of 1:1,000, Chagas-Flow ATE demonstrated high accuracy to discriminate serum samples from the NT, BZ2 and BZ3 groups. Furthermore, was demonstrated moderate accuracy to segregate serum samples from BZ1, with these same antigens. Overall, most serum samples from treated patients were negative in Chagas-Flow ATE, while all serum samples from these same patients were reactive when tested by ELISA. Furthermore, our proposal for this study will be to improve Chagas-Flow ATE into a multiplex platform for universal and genotype-specific diagnosis, and for follow-up monitoring of chronic CD. Thus, Chagas-Flow ATE proved to be a promising method for follow-up monitoring of chronic CD patients.

Financial support: CNPq, FAPEMIG, CAPES.

Keywords: Chagas disease; Trypanosoma cruzi; Flow Cytometry.

2632 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Evaluation of the Role of Extracellular Vesicles in Immune System Modulation in Plasmodium vivax-Infected Patients

RODRIGUES, B.C.M.; CORDEIRO, L.P.B.; ROSA, M.J.G.; GOMES, C.B.S.; DE LACERDA, L.B.; JUNQUEIRA, C.F.. FUNDAÇÃO OSWALDO CRUZ, BELO HORIZONTE - MG - BRASIL.

Anemia and thrombocytopenia are the most frequent complications in malaria caused by *Plasmodium vivax*, with this species being responsible for high morbidity, especially in children and pregnant women living in endemic areas. Although these immunopathologies have a multifactorial nature, it's known that exacerbated inflammation is an important component in the development of these clinical manifestations in vivax malaria. Furthermore, exacerbated inflammation contributes to increased release of extracellular vesicles (EVs), which can act as mediators of cell-to-cell communication and as modulators of immune system activation through the transfer of cellular mediators, pathogen-associated molecular patterns, damage-associated molecular patterns, and microRNAs. In a previous study by our group, EVs from the plasma of individuals infected with *P. vivax* were analyzed, and two populations of EVs were identified as increased in patients presenting with thrombocytopenia. One of these populations had monocytic origin, and the other consisted of opsonized EVs. These changes suggest a role for EVs in communication between monocytes and a relationship with an antibody-dependent response, possibly associated with this clinical manifestation. Considering the preliminary nature of the previous results obtained, an extensive study is proposed aiming at the characterization and evaluation of the role of EVs in the modulation of the immune response during vivax malaria. To this end, the following are proposed in this work: the characterization of phenotypic markers by multiparametric nano-flow cytometry, the identification of antigen-antibody complexes, and the characterization of coding and non-coding RNA contents in EVs isolated from the plasma of infected individuals with or without clinical manifestations. Furthermore, we will assess the internalization of these vesicles and their capacity to induce a response in the effector cells of interest.

Keywords: Extracellular vesicles; Monocytes; Malaria Vivax.

2633 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Development and evaluation of the protective potential of an mRNA vaccine against *Plasmodium* spp.

ROSA, M.J.G.¹; RODRIGUES, B.C.M.¹; CORDEIRO, L.P.B.¹; GOMES, C.B.S.¹; DE LACERDA, L.B.¹; TEIXEIRA, S.M.R.²; JUNQUEIRA, C.F.¹. 1. FIOCRUZ, BELO HORIZONTE - MG - BRASIL; 2. UFMG, BELO HORIZONTE - MG - BRASIL.

As demonstrated during the COVID-19 pandemic, mRNA vaccines have become a promising alternative to conventional vaccination methods due to their rapid production, high versatility, and ability to induce strong cellular and humoral immune responses. Beyond their proven effectiveness against viral infections, RNA vaccines hold significant potential for preventing infections caused by parasitic protozoa, particularly intracellular parasites, where effective protection depends on a robust cellular immune response. Among these pathogens are *Plasmodium* species, the causative agents of malaria. Recently, two vaccines based on the recombinant circumsporozoite protein (CSP) of *Plasmodium falciparum* were approved and have shown promising results in African countries. However, since most malaria cases in Brazil are caused by *Plasmodium vivax*, these vaccines do not offer protection against the most common form of malaria in the country. To address this gap, our group has been investigating two recombinant proteins as vaccine candidates against *P. vivax*: the circumsporozoite protein (PvCSP) and the 60S ribosomal protein L30, the latter identified through immunoproteomic studies as a novel antigen capable of eliciting a strong cellular immune response. Preliminary data from our group indicate that mRNA formulations encoding PvCSP induce robust humoral and cellular immunity. In this project, we aim to test immunization protocols that alternate doses of recombinant protein vaccines with doses of corresponding mRNA formulations. Additionally, we will evaluate protocols using mRNA formulations encoding both antigens and compare the protective efficacy of these approaches in mouse models of malaria infection. Keywords: *Plasmodium vivax*; Malaria; mRNA Vaccine.

2635 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Extracellular Vesicle Profile Associated with Phenotypic-Functional and Clinical Parameters as a Predictive Tool for Prognosis in Endometriosis

SILVESTRINI, M.M.A.¹; FRADICO, J.R.B.²; CARVALHO, N.O.²; PASCOAL-XAVIER, M.A.¹; PERUHYPE-MAGALHÃES, V.¹; JÚNIOR, J.O.D.A.F.³; SCHETTINO, K.D.C.³; BARBOSA, L.M.P.⁴; NEVES, A.V.B.¹; MARTINS-FILHO, O.A.¹; QUEIROZ, F.R.⁵; SALLES, P.G.O.⁵; BRAGA, L.D.C.⁵; TORRECILHAS, A.C.T.⁶; CARVALHO, A.T.¹. 1. FIOCRUZ MINAS, INSTITUTO RENÉ RACHOU, BELO HORIZONTE - MG - BRASIL; 2. INSTITUTO RENÉ RACHOU FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 3. HOSPITAL FELÍCIO ROCHO, BELO HORIZONTE - MG - BRASIL; 4. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 5. INSTITUTO MÁRIO PENNA, BELO HORIZONTE - MG - BRASIL; 6. UNIVERSIDADE FEDERAL DE SÃO PAULO, BELO HORIZONTE - MG - BRASIL.

Endometriosis is a chronic, multifactorial, neuroinflammatory gynecological disease that affects approximately 10% of the global female population. It is associated with pelvic pain, dysmenorrhea, dyspareunia, and infertility, and is often diagnosed late through invasive procedures such as laparoscopy. Given this, the need for less invasive and more accessible diagnostic methods, such as liquid biopsy, becomes evident. In this context, extracellular vesicles (EVs) stand out due to their ability to transport proteins, lipids, cytokines, chemokines, and RNAs (including microRNAs), positioning them as prognostic biomarkers and therapeutic targets. This is a cross-sectional, descriptive, and exploratory study conducted at the René Rachou Institute/FIOCRUZ Minas, in partnership with Felício Rocho Hospital (Belo Horizonte – MG, Brazil). Peripheral blood samples were analyzed from 60 women with endometriosis and 30 women without the disease (control group, including asymptomatic individuals and those with pelvic pain unrelated to endometriosis). EVs were isolated using ultracentrifugation and size-exclusion chromatography, and characterized through nanoparticle tracking analysis and CD63 marker positivity by ELISA. The profile of circulating immune mediators was assessed using a multiplex assay for detecting chemokines, cytokines, and growth factors. The results showed similar EV size and concentration profiles between groups. However, a significant increase in chemokines (CCL5, CCL11, CXCL12) and inflammatory cytokines (IL-12p70 and TNF- β) with a pro-inflammatory profile was observed in patients with endometriosis versus the control group. This study aims to contribute to developing a predictive biomarker panel accessed through liquid biopsy to support clinical and post-therapeutic monitoring, improving patient quality of life and reducing treatment costs. **Funding sources:** FIOCRUZ, CNPq, CAPES, FAPEMIG, and Felício Rocho Hospital. Keywords: endometriosis; extracellular vesicles; biomarkers.

2636 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Evaluation of T lymphocyte populations in symptomatic and asymptomatic individuals infected with *Plasmodium vivax*

GUNDERMAN, V.H.G.¹; COSTA, C.M.¹; ALMEIDA, G.G.¹; DE OLIVEIRA, G.P.C.¹; FIGUEIREDO, M.M.²; PEREIRA, D.³; TADA, M.S.³; GAZZINELLI, R.T.¹; ANTONELLI, L.¹. 1. INSTITUTO RENÉ RACHOU/FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE DO ESTADO DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. CENTRO DE PESQUISA EM MEDICINA TROPICAL, PORTO VELHO - RO - BRASIL.

Individuals infected with *Plasmodium vivax* may develop the asymptomatic (ASY) form of the infection. Those individuals have very low parasitemia, usually undetectable by microscopy, becoming parasite reservoirs and perpetuating transmission. The cellular immune response against *P. vivax* involves a complex interaction of conventional and unconventional T cells, including $\gamma\delta$ T cells and invariant Natural Killer T (iNKT) cells, which act at different stages of the parasite's life cycle. This project aims to define the composition of conventional and unconventional T cell populations of ASY and symptomatic (SY) *P. vivax*-infected individuals living in the Brazilian Amazon. Samples were analyzed using a 28-parameter flow cytometry panel to characterize T cells – conventional (CD4⁺ and CD8⁺) and unconventional (expressing TCR $\gamma\delta$ or TCRV α 24: iNKT) – as well as their memory (CD45RA, CCR7, CD28, CD95) and activated (CD38, HLA-DR) subpopulations. SY and ASY showed higher frequencies of central memory (CM) Tfh cells and stem cell-like memory CD8⁺ T (TSCM) cells compared to non-infected controls (CTL). Specifically, SY exhibited increased activated CD8⁺, TSCM CD4⁺ T cells and effector memory (EM) Tfh cells, as well as a reduction in naïve CD4⁺ T cells compared to ASY and CTL, along with a higher frequency of activated CD4⁺ T cells compared to CTL. In contrast, ASY exclusively showed a lower frequency of naïve CD8⁺ T cells compared to CTL and SY, a higher frequency of EM Tfh cells compared to CTL, though still lower than SY, and a higher frequency of terminal effector memory CD8⁺ T (TEMRA) cells compared only to CTL. Our data suggest that SY is characterized by an expansion of activated and memory T cell subpopulations. Meanwhile, ASY exhibit a regulated immune response with effector profile. Unsupervised analyses and correlations between immunological and clinical data will be carried out for a complete understanding of the ASY status of *P. vivax* infection.

Keywords: *Plasmodium vivax*; Asymptomatic infection; T lymphocytes.

2639 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Comprehensive Profiling of B Lymphocyte Subsets in Asymptomatic and Symptomatic *Plasmodium vivax* Infections

DE OLIVEIRA, G.P.C.¹; COSTA, C.M.¹; ALMEIDA, G.G.¹; GUNDERMAN, V.H.G.¹; FIGUEIREDO, M.M.²; PEREIRA, D.³; TADA, M.S.³; GAZZINELLI, R.T.¹; ANTONELLI, L.¹. 1. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE ESTADUAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. CENTRO DE PESQUISAS EM MEDICINA TROPICAL, PORTO VELHO - RO - BRASIL.

Plasmodium vivax is the most prevalent malaria-causing species in Brazil. Infections caused by this parasite can result in asymptomatic (ASY) cases, characterized by low parasitemia, persistent infection, and the potential to serve as reservoirs. Although *P. vivax* does not induce long-lasting immune protection, B lymphocytes (LB) still play a crucial role in short- to medium-term immune responses by helping to control the infection through various mechanisms, including antibody production. In our study, we developed a 28-parameter flow cytometry panel to characterize LB subpopulations and identify markers potentially associated with ASY infection (n = 20), compared to symptomatic (SY; n = 15) and uninfected controls (CTL; n = 11). Our results showed that both ASY and SY exhibit higher frequencies of Activated Memory LB (AM), both class-switched and unswitched, compared to CTL. However, SY cases demonstrated a greater expansion of class-switched AM cells relative to ASY. SY also had more plasmablasts than CTLs, a difference not observed in ASY cases. Conversely, total memory LB were more abundant in CTL and ASY than in SY individuals. Preliminary unsupervised analysis using FlowSOM revealed that ASY individuals possess a higher frequency of Classical Memory LB (CM), whereas SY individuals show a predominance of AM cells. As a next step, we plan to further investigate LB subpopulations using unsupervised clustering to evaluate markers of costimulation (CD40), activation/maturation (TACI, BAFFR, CD71), migration (CXCR3, CXCR5), Fc receptors (CD16, CD23, CD64), proliferation (Ki67), and atypical markers (CD1c, CD11c, CD123). Additionally, we will assess immune profiles in ASY and SY post-treatment. Our findings suggest distinct LB dynamics between ASY and SY *P. vivax* infections, with implications for immune regulation and potential targets for future interventions. Further analysis may reveal mechanisms underlying ASY carriage and support strategies to eliminate malaria.

Keywords: *Plasmodium vivax*; Asymptomatic infections; B lymphocytes.

2648 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Antigenicity assessment of Plasmodium vivax antigens using Enzyme-Linked Immunosorbent Assay and Western Blotting platforms

RODRIGUES, J.V.G.; DE LACERDA, L.B.; GOMES, C.B.S.; JUNQUEIRA, C.F.. FIOCRUZ, BELO HORIZONTE - MG - BRASIL.

Malaria remains a major global health concern, with 263 million cases reported worldwide in 2023. In Brazil, 131,224 cases were recorded, the majority due to *Plasmodium vivax*. Uniquely, *P. vivax* infects reticulocytes—immature erythrocytes that retain parts of the protein translation machinery. This characteristic enables infected cells to express HLA class I molecules, activating CD8+ cytotoxic T cells, which in turn recognize infected reticulocytes and release cytotoxic granules such as granzyme B, leading to parasite elimination.

An immunopeptidomics study identified 573 *P. vivax*-specific peptides presented by HLA-I molecules, many of which originated from essential proteins, including ribosomal components. Among them, the ribosomal protein 60S L30 was selected for further evaluation as a vaccine candidate due to its high conservation and expression across parasite stages. Despite its biological importance, little is known about the immunogenicity of this protein, especially in relation to humoral immune responses.

This study aimed to evaluate the IgG antibody response to the recombinant *P. vivax* L30 protein through an optimized indirect ELISA and Western blotting. Sera from non-endemic and endemic healthy individuals, as well as from patients infected with *P. vivax* and *P. falciparum*, were analyzed. ELISA results revealed unexpectedly higher antibody titers in sera from *P. falciparum*-infected individuals, indicating possible cross-reactivity, likely attributable to structural homology between the L30 proteins of both species. Western blotting further confirmed antibody recognition of the L30 protein in sera from both *P. vivax* and *P. falciparum* infections, as well as the presence of the L30 protein in erythrocyte extracts from *P. falciparum*-infected samples.

These findings indicate the antigenic potential of the *P. vivax* L30 protein and support its continued evaluation as a cross-species vaccine target.

Keywords: Malaria;Antigenicity;ELISA.

2657 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

FECAL MICROBIOTA TRANSPLANTATION MODULATES THE ESTROGEN-GUT-BONE AXIS AND BONE HOMEOSTASIS IN POSTMENOPAUSAL OSTEOPOROSIS

DOS SANTOS, A.C.P.M.; FERREIRA, L.M.A.; DE OLIVEIRA, P.A.; OLIVEIRA, S.N.; LUCAS, L.M.B.; SANTOS, B.M.D.A.; SILVA, M.E.F.; QUEIROZ JUNIOR, C.; ARIFA, R.D.D.N.; TEIXEIRA, M.M.; DE SOUZA, D.D.G.; FAGUNDES, C.T.; MADEIRA, M.F.M.. UFMG, BH - MG - BRASIL.

Postmenopausal osteoporosis is a bone resorption disease associated with altered differentiation of myeloid cells and osteoclasts. During osteoporosis, increased intestinal permeability promotes bacterial translocation and exacerbates systemic inflammation. In this context, fecal microbiota transplantation (FMT) has emerged as a potential tool to mitigate these effects. This study aimed to investigate the role of the estrogen-gut-bone axis in bone metabolism and the regulatory potential of FMT. We used a postmenopausal osteoporosis model induced by ovariectomy (OVX) in female C57BL/6J mice (6–8 weeks old) (CEUA: 162/2024). After 21 days, OVX mice showed weight gain and uterine atrophy. Additionally, bacterial translocation to the liver and changes in fecal microbiota composition were observed. Histological analysis showed increased inflammatory infiltrate and structural changes in the colon, along with reduced mucus production. The intestinal permeability assay revealed greater Evans Blue translocation to the colon, liver, and plasma in OVX mice. These anatomical and microbiota alterations were associated with immune dysregulation. Flow cytometry revealed no increase in inflammatory cells in the colon (macrophages, neutrophils, and dendritic cells), possibly linked to altered myeloid differentiation in bone marrow, with high numbers of macrophages and pre-osteoclasts. To modulate these changes, we implemented an FMT model using microbiota from healthy donors. Mice were assigned to Sham or OVX groups receiving saline, antibiotics (ATB) or FMT. ATB and FMT groups received antibiotics prior to administration of either saline or fecal microbiota via gavage. OVX-FMT mice showed no significant changes in weight or bacterial translocation. However, colon alterations persisted in OVX and ATB groups and were not reversed by FMT. These findings suggest that osteoporosis involves inflammatory and microbiota changes, and that FMT holds regulatory potential in this context.

Keywords: Osteoporosis;gut microbiota;myeloid cells.

2661 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Annexin A1 combined immunochemotherapy is highly effective for treatment of tegumentar leishmaniasis

RICOTTA, T.Q.N.; DOS SANTOS, L.M.; DE OLIVEIRA, L.G.; CARVALHO, A.F.S.; GOULART, G.A.C.; DE SOUSA, L.P.; FERNANDES, A.P. UFMG, BELO HORIZONTE - MG - BRASIL.

Leishmaniasis, a neglected disease, presents a significant global health challenge due to its impact on millions and the high costs and logistical difficulties of current treatments. Oral and topical therapies are promising alternatives, especially for cutaneous leishmaniasis (CL), offering reduced systemic effects and improved patient adherence. We explore the therapeutic potential of AnxA1, known for its anti-inflammatory and pro-resolution properties. We investigated AnxA1's efficacy for treatment of CL using the peptide Ac2-26 (AC) alone or combined with miltefosine (Milt). *L. amazonensis*-infected mice received: topical AC, IP AC, suboptimal oral Milt, a combination of Milt and AC (both routes), or full-dose oral Milt (FDM). All treated groups showed reduced edema compared to untreated mice. Notably, the combined AC and Milt resulted in smaller edema than single-drug treatments. By week six, lesions in the combined AC and Milt and FDM groups were no longer visible. Ulceration analysis revealed that while some treated groups showed ulceration, the area was significantly smaller at later time points compared to untreated mice, indicating reduction of both edema and ulceration. Parasite load was lower in mice receiving topical and IP AC than in untreated controls. The combined therapy groups exhibited a lower parasite load than single-drug treatments, with the topical AC+Milt group and the FDM group showing no detectable parasites. Regarding cytokine production, no difference was observed in IL-10 production. However, animals receiving AC showed lower INF- γ levels. Lymph node cell profiles remained largely unchanged, except for macrophages: combined IP AC and Milt treatment led to a lower number of these cells, while the higher Milt dose resulted in an increased number. Our findings highlight Ac2-26 as a promising therapeutic agent for leishmaniasis, particularly its potential in combination with existing drugs like Milt for immunotherapy approach.

Keywords: leishmania;annexin a1;Ac2-26.

2669 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

FPR2 Modulates Early Immune Responses and Parasite Burden During Leishmania amazonensis Infection

RICOTTA, T.Q.N.; DE OLIVEIRA, G.; DOS SANTOS, L.M.; DE SOUSA, L.P.; CARVALHO, A.F.S.; FERNANDES, A.P. UFMG, BELO HORIZONTE - MG - BRASIL.

Formyl Peptide Receptor 2 (FPR2) has been known to be involved in the regulation of host immune responses and inflammation. To investigate its role in cutaneous leishmaniasis, female C57BL/6 wild-type (WT) and FPR2-deficient (KO) mice were infected with 1×10^5 *Leishmania amazonensis* promastigotes in the left hind footpad. Lesion development was tracked weekly, and parasite load was quantified at week 9 using limiting dilution. Although lesion sizes remained comparable between groups, KO mice exhibited significantly reduced parasite burdens at early infection stages. Cytokine profiling of draining lymph nodes showed increased IFN- γ and decreased IL-10 in KO animals, reflecting a more pro-inflammatory Th1-skewed response. Flow cytometry analysis of lymph nodes cells revealed higher counts of CD4⁺ and CD8⁺ T cells in KO mice early on, whereas WT mice demonstrated enhanced recruitment of dendritic cells, macrophages, and neutrophils at later time points. Administration of the pro-resolving peptide Ac2-26 led to diminished lesion development and parasite burden in both WT and KO groups, without substantial changes in cellular immune profiles. Western blot assays showed short-lived reductions in phosphorylated ERK and Annexin A1 expression in KO mice during early infection, followed by normalization over time. These findings suggest that FPR2 modulates early immune activation and parasite control during *L. amazonensis* infection, and that targeting resolution pathways may offer novel therapeutic approaches for leishmaniasis.

Keywords: Leishmaniasis;FPR2;Ac2-26.

2670 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Analysis of soluble factors indicates that the HLA-A*02 haplotype may be associated with a distinct immunological profile in individuals with long COVID

DIAS, L.C.C.; RIBEIRO, Á.L.; FERREIRA, G.M.; CLARINDO, F.A.; RETES, H.M.; ROCHA, V.; TEIXEIRA, C.W.; DE PONTES, L.G.; DOS SANTOS, E.A.S.; PEREIRA, C.L.D.; FÉLIX, M.E.M.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Long COVID (LC) is generally defined as a multisystem syndrome with symptoms that persist for 3 months or more after acute COVID-19. LC symptoms range from mild to severe and several organs and systems can be affected, especially the nervous system. It is estimated that 10% of those infected with SARS-CoV-2 suffer from LC, however, the pathophysiological mechanisms and therapeutic strategies for this disease remain unknown. Considering the importance of MHC-I antigen presentation in antiviral responses and the long-term effect of MHC-I haplotypes in variations for the development and manifestations of long COVID, this study aimed to evaluate the profile of the immune response against SARS-CoV-2 in individuals with long COVID who are carriers and non-carriers of the HLA-A*02 haplotype. For this, whole blood samples were collected from individuals without long COVID (WLC = 60) and those with long COVID who presented symptoms from 6 to 18 months (LC = 227). The plasma samples were collected and used to assess 48 soluble factors using the Luminex methodology and graphical analyses were carried out using GraphPad Prism 8.0 and Cytoscape softwares. Our data revealed that individuals with long COVID carrying the HLA-A*02+ allele had significantly increased plasma levels of IL-7, IL-12(p70), and IL-13, compared to individuals HLA-A*02- with long COVID. We also observe a loss of strong correlations between the analyzed parameters of individuals with long COVID when compared to individuals without long COVID, for HLA-A*02+ individuals there was a loss of 21 strong correlations, whereas for HLA-A*02- individuals 59 strong correlations were lost. Altogether, these results point to a potential role of immunogenetics factors in modulating the immune response in long COVID that can possibly lead to a distinct immunological profile characterized by elevated Th1 and Th2 activity. However, further research focusing on cellular immunity is required to validate such observations.

Keywords: Long COVID;MHC-I;SARS-CoV-2.

2675 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

MURINE CORONAVIRUS INFECTION ALTERS THE ADAPTIVE IMMUNE RESPONSE WITH EXACERBATED INFLAMMATION AFTER *Candida albicans* INFECTION, AFFECTING LUNG FUNCTION

LUCAS, L.M.B.; SILVA, M.E.F.; OLIVEIRA, S.N.; BARBOSA, J.P.P.; DIAS, A.D.A.S.; QUEIROZ JUNIOR, C.; DA GRAÇA-FILHO, R.V.; SOUZA, J.L.N.; SANTOS, B.M.D.A.; RUSSO, R.D.C.; TEIXEIRA, M.M.; ARIFA, R.D.D.N.; DE SOUZA, D.D.G.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

COVID-19 is an infectious disease caused by the SARS-CoV-2 virus that has led to a high number of deaths and comorbidities. The persistence of symptoms after COVID-19 infection has been termed long COVID, with immunological alterations among the most notable symptoms. Therefore, our objective was to investigate whether changes in the activation profile of T lymphocytes induced by murine coronavirus infection interfere with the host's susceptibility to infection by *Candida albicans*. Initially, female C57BL/6 mice were infected intranasally with MHV-A59 at an inoculum of 1×10^4 PFU/animal, and after 7 days, they were subjected to *C.albicans* infection at an inoculum of 1×10^6 yeasts/animal via intratracheal administration, with euthanasia occurring after 2 days. Flow cytometry showed an increase in CD8+ T lymphocytes and inhibition of regulatory T lymphocytes only in the group subjected to both infections. Histologically, the same group presented a more robust inflammatory infiltrate compared to the control groups. At a later stage of infection, with euthanasia occurring 23 days after MHV-A59 infection and 2 days after *C.albicans* infection, we observed the same T lymphocyte profile as before, with a predominance of effector T lymphocytes and inhibition of regulatory T lymphocytes in the group subjected to both infections. To assess the long-term impact on lung function from both infections, euthanasia was performed 30 days after MHV-A59 infection and 9 days after *C.albicans* infection. Through spirometric analyses, both virus-infected groups showed increased pulmonary compliance parameters and decreased pulmonary resistance parameters in the group subjected to both infections, consistent with an obstructive lung disease pattern. The results suggest that coronavirus has the potential to alter the immune response via lymphocytes, enhancing the effector response without proper regulation, thereby compromising lung function.

Keywords: Long COVID;immunological alterations;post-COVID susceptibility.

2678 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The role of the Ffar2 receptor in immune modulation during *L. major* infection

PEIXOTO, J.M.; MOREIRA, F.C.; FARIA, A.M.C.; MAIOLI, T.U.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Intestinal microbiota contributes to immune homeostasis through production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. Metabolites with energetic, epigenetic, and immunomodulatory functions, acting via histone deacetylase (HDAC) regulating receptors like GPR43 (Ffar2). Ffar2, expressed in various immune cells, including neutrophils, T cells, B cells, and ILC3, affecting processes like chemotaxis, cytokine production, and lymphocyte differentiation. Ffar2 has involved in diseases like colitis and bacterial infections, its role in parasitic diseases remains unclear. Leishmaniasis, a neglected tropical-parasitic disease that affect over 12 million people globally, with immune-mediated infection control or progression. CD4⁺ and CD8⁺ T cells and neutrophils contribute to parasite clearance (via IFN- γ and macrophage activation) or exacerbate pathology, cytotoxicity and IL-4/IL-10 production, in *L. major* infection. Immunomodulatory role of Ffar2 in this context is unknown. Project aims to investigate if Ffar2 influences progression of *L. major* infection exploring interactions between immune responses and gene regulation. Objective is to identify potential therapeutic targets based on Ffar2 signaling and SCFA modulation. Study analyzes bulk RNA-seq public transcriptomic data (GEO: GSE185253) comparing *L. major*-infected mice with and without Ffar2 expression. Differentially expressed genes were correlated with Ffar2 using Spearman analysis and visualized with *ggplot2* and *heatmap*. Gene networks were built with *igraph*, and centrality metrics identified hub and bridge genes. Genes identified as hubs and bridges include *Wdr83*, *Parp11*, *Rab3ip*, *Ccdc71*, and *Rspo3*. *Rspo3*, *Rab3ip*, and *Wdr83* and may modulate immune responses via inflammation induction and vesicle trafficking; *Parp11* may have regulatory role, while function of *Ccdc71* still unclear. Findings suggest Ffar2-related pathways could be explored as targets for leishmaniasis immunomodulation.

Keywords: Ffar2;Leishmaniasis;Short chain fat acids.

2685 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Maternal folic acid restriction associated with Zika virus infection during pregnancy in a murine model compromises fetal development, leading to physiological and neurobehavioral changes in adulthood

AZEVEDO, L.M.S.; DINIZ, L.N.A.; OLIVEIRA, S.N.; SANTOS, B.M.D.A.; RIBEIRO, R.D.S.; RIBEIRO, N.D.A.R.; TEIXEIRA, N.W.; DOS SANTOS, A.C.P.M.; DIAS, M.V.; AMARAL, B.S.; SILVA, M.E.F.; TEIXEIRA, D.C.; SANTOS, F.R.D.S.; COSTA, G.M.J.; BONAVENTURA, D.; DE OLIVEIRA, A.C.P.; COSTA, V.V.; TEIXEIRA, M.M.; DE SOUZA, D.D.G.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Zika virus (ZIKV) is an arbovirus associated with neurological complications, microcephaly and fetal anomalies. This group of fetal malformations associated with ZIKV is known as Congenital Zika Syndrome. In 2015, Brazil experienced an outbreak of ZIKV where it was observed that clinical signs were aggravated in regions of social vulnerability and malnutrition, which possibly related to the low intake of important nutrients for fetal formation, like folic-acid, fundamental in the formation of the nervous system, spot of ZIKV tropism. Thus, the objective of this study was evaluating the effect of the absence of folic-acid in the pre and postnatal period of the offspring of mice infected with ZIKV. Pregnant female C57BL/6 mice (CEUA/UFMG-319/2022) previously subjected to control diet and folic-acid-free diet received PBS or ZIKV on embryonic day (ED) 5.5, obtaining the groups: control diet Ni and ZIKV (Ni; C/ZIKV); folic-acid-free-diet Ni and ZIKV (AF-; AF-/ZIKV). Fetal tissues were collected on DE 15.5 or the offspring were waited for birth and accompanied for 12 weeks. In the prenatal phase, a reduction in fetal weight, increase in the concentration of inflammatory mediators, viral load and a decrease in the recruitment of macrophages and neutrophils in the placenta were observed in AF-/ZIKV. In the brain, increase in the concentration of inflammatory mediators and macrophage recruitment were observed, additionally to inadequate morphological development in AF-/ZIKV. In the postnatal period, the pups presented brain and ocular malformations, cardiac dysfunction, bone loss and reproductive impairment, in addition to behavioral changes such as memory loss, anxiety and hyperactivity in AF-/ZIKV. These findings suggest increased maternal immune activation in the face of ZIKV infection associated with maternal folic acid deficiency during pregnancy, reflecting in the development of offspring into adulthood.

Keywords: Zika virus;Folic Acid;Maternal Immune Activation.

2687 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

EVALUATION OF HLA-A*02-RESTRICTED IMMUNODOMINANT PEPTIDES IN BRAZILIAN TEMPORAL LINEAGES OF THE VIRUS *Orthobunyavirus oropoucheense*

FÉLIX, M.E.M.; RIBEIRO, Á.L.; DIAS, L.C.C.; MENDES, G.E.R.; DA FONSECA, F.G.; DOS REIS, J.G.A.C.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Oropouche fever is an arbovirus disease caused by the *Orthobunyavirus oropoucheense* virus (OROV). In recent years, the disease has become a cause for concern due to the increase in cases in Brazil. By May 2025, the number of confirmed cases represents 73% of the total cases for 2024, with 4 deaths confirmed in Brazil, indicating the need to search for therapeutic and vaccinal strategies to control viral circulation in the country. Considering that the CD8⁺T cell response is crucial for an effective antiviral immune response, this work aims to evaluate the HLA-A*02-restricted peptide signature from OROV in a temporal manner. For that, viral polyprotein sequences isolated during the OROV outbreaks of 2016, 2020, and 2024 in Brazil were obtained from the NCBI nucleotide database and used to predict HLA-A*02-restricted peptides in the NetCTL 1.2 software. Peptides with a combined score >0.50 were selected for further analysis. Bioinformatics tools were also used to evaluate cytokine induction by the peptides. Our results indicate a stability of the virus among the three temporal lineages analyzed regarding the number of peptides (n≈267) and prediction score value. However, a greater immunogenic potential was observed in the M segment, with 7.7% of the peptides considered epitopes, compared to 6.7% of S and 6.4% of the L segment. Additionally, the induction of cytokines such as IL-10 and IFN-γ was greater for the peptides of this segment, highlighting the M segment as a promising target to trigger a balanced antiviral CD8⁺T cell response. A higher percentage of peptides exclusive to the 2016 lineage was also noted in the M segment (34%) compared to the L (3.9%) and S (0%) segments, indicating a potential 'hot-spot' for immune evasion and pointing towards the need to further assess peptides from this polyprotein, using *in silico* and *in vitro* tools to identify immunogenic and conserved peptides that could be used for the development of vaccinal and therapeutic platforms.

Keywords: Oropouche fever;immunogenicity;epitopes.

2688 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Study of the role of inflammatory monocytes in the control of experimental *Leishmania major* disease in mice

PAIXAO, P.H.D.M.¹; BARBOSA, V.²; MURTA, G.K.T.¹; DE SOUZA, I.A.¹; GONÇALVES, R.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. FACULDADE ATENAS, SETE LAGOAS - MG - BRASIL.

Leishmaniasis is a group of diseases caused by protozoan parasites of the genus *Leishmania*, which are obligate intracellular digenetic parasites that primarily infect macrophages in vertebrate hosts. Although macrophages are considered key cells in controlling the infection, our studies have shown that once infected, they become unable to eliminate the parasite *in vitro* models, complicating the understanding of how natural infection is controlled. We have recently demonstrated that inflammatory monocytes (GR1/Ly6C⁺ CCR2⁺) can control experimental infection in mice, being the first cells to migrate to the site of infection and eliminate the parasites. This migration is CCR2-dependent, and C57BL/6 CCR2 knockout mice exhibit increased parasite burden and lesion size, indicating impaired infection control. The aim of this study was to evaluate the role of inflammatory monocytes in controlling experimental leishmaniasis in mice. To this end, we used C57BL/6 mice, C57BL/6 CCR2 knockout mice, and a parabiosis model in which these two strains were surgically joined. The right hind paw of CCR2 knockout mice was infected with *Leishmania major*, and parasite burden, lesion size, and monocyte involvement during infection were analyzed. We observed the restoration of CCR2⁺ monocytes in the circulation of CCR2 knockout mice in parabiosis, along with reduced parasite burden and lesion size. These results suggest that inflammatory monocytes play a crucial role in controlling *L. major* infection in mice.

Keywords: *Leishmania major*;Inflammatory monocyte;CCR2.

2689 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Interferon alfa and beta receptor confers resistance against *Neospora caninum* infection in mice

MIRANDA, V.D.S.; FRANÇA, F.B.F.; SILVA, M.F.; MINEO, J.R.; MINEO, T.W.P.. UFU, UBERLÂNDIA - MG - BRASIL.

Neospora caninum is an intracellular protozoan that is the causative agent of neosporosis, a disease relevant to the veterinary field for causing neuromuscular disease in dogs and repeated abortions in ruminants. In the present moment, there are no therapies that are able to induce sterile cure against the infection and prevention strategies rely only in environmental management. In this context, we aimed to determine the protective role of the Interferon alpha and beta receptor (IFNAR) during an experimental infection with *N. caninum*. We performed *in vivo* experiments with genetically deficient mice (*Ifnar*^{-/-}). Those mice, in direct comparison with wildtype (WT) controls, presented reduced survival against the infection, associated with increased parasite load and inflammation in different tissues. In agreement, we observed that the absence of the IFN-I receptor led to a significant reduction in crucial inflammatory mediators that control the infection, namely IL-12, IFN-γ and nitric oxide. Together, our results showed that IFNAR is a central molecule that mediates host resistance against *N. caninum* by controlling acute and chronic parasite replication, through the induction of an effective Th1 immune response against this protozoan parasite.

Financial support: FAPEMIG, CAPES, CNPq

Keywords: IFNAR;Neosporosis;Innate Immunity.

2690 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

IN SILICO ANALYSIS OF Orthopoxvirus Monkeypox EPITOPES: UNRAVELING TH1/TH2 MECHANISMS RELATED TO IMMUNOMODULATION OF A REEMERGING PATHOGEN

MENDES, G.E.R.¹; FÉLIX, M.E.M.¹; FERNANDES, J.G.C.D.²; DIAS, L.C.C.¹; RIBEIRO, Á.L.¹; STANCIOLI, E.F.B.¹; DA FONSECA, F.G.¹; DOS REIS, J.G.A.C.¹. 1. FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. SANTA CRUZ STATE UNIVERSITY, ILHÉUS - BA - BRASIL.

Mpox is an important disease caused by the *Orthopoxvirus Monkeypox* (MPXV), which is divided into two clades. These clades are characterized by variable symptoms and mortality rates. Considering its pandemic potential, mpox was declared a public health emergency of international concern by the WHO in 2022. By 2024, thousands of cases had been identified. The pathogenesis of MPXV is significantly influenced by the host's immune response, especially its capacity to evade the lymphocytic response. Therefore, studies on the peptide signature of MPXV and its implications for the T-cell response are crucial for a better understanding of both the host immune response to MPXV and the pathogenicity of the virus. This research uses vaccinomics and computational methods to identify and describe linear CD8+ T cell epitopes from *Monkeypox*. MPXV reference sequences for clades I and II were obtained from the NCBI Nucleotide database and used to predict T-cell peptides using the NetCTL 1.2 predictor. A total of 431688 peptides were initially selected for HLA-A*01, HLA-A*02, HLA-A*03, and HLA-A*24, but only peptides with a Combined Score (CS) of 0.75 were maintained. Given our results of CS values for peptides restricted to HLA-A*01, these sequences were chosen for further analysis. Peptides restricted to HLA-A*02 were also included due to the high frequency of this haplotype in the world population. A total of 8035 peptides were evaluated *in silico* for their potential to induce regulatory (IL-4, IL-5, IL-10, IL-13) and pro-inflammatory (IL-2, TNF-α, IFN-γ, IL-17) cytokines, as well as IgE. Preliminary results indicated a large number of peptides with the potential to induce IL-4 and IL-13 and a small number of peptides with the potential to induce IL-5, IL-10, and IL-17, suggesting new mechanisms for MPXV that combine the Th1 and Th2 pathways. Further studies include describing the *in vitro* reactivity of MHC-I and the physicochemical properties of these peptides.

Keywords: Cytokines;Monkeypox;Vaccinomics.

2698 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Eosinophil Deficiency Exacerbates Lung Pathology and Gut Dysbiosis in Experimental Toxoplasmosis

DE FREITAS, G.M.¹; SANTANA, L.F.D.²; SILVA, L.P.²; SANTOS, L.L.O.²; OLIVEIRA, F.B.R.²; MELO, N.²; NONATO, A.²; DE MEIRA, T.A.²; BARBOSA, C.L.N.²; CASSALI, G.D.³; MARTINS, F.D.S.⁴; RICCI, M.F.²; MACHADO, F.S.². 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. DEPARTAMENTO DE BIOQUÍMICA E IMUNOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS (ICB), UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL; 3. DEPARTAMENTO DE PATOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS (ICB), UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL; 4. DEPARTAMENTO DE MICROBIOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS, (ICB), UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL.

Toxoplasma gondii is the etiological agent of toxoplasmosis, a globally distributed infection, in which one-third of the human population harbors chronic latent disease. Although typically asymptomatic in immunocompetent hosts, it can trigger severe immunopathological outcomes in congenital transmission and immunocompromised patients. These manifestations may lead to fatal outcomes, particularly in congenital transmission and immunocompromised patients, and are linked to a dysregulated host immune response, although the mechanisms remain poorly understood. In this context, eosinophils have emerged as key immunomodulators, yet their role in toxoplasmosis pathogenesis remains unexplored. Herein, we investigated the role of eosinophils in toxoplasmosis pathogenesis. Female BALB/c (WT) and Δ dblGATA-1 knockout (KO) (eosinophil-deficient) mice were infected (IF) or not intraperitoneally with 20 cysts of *T. gondii* (ME49 strain). We monitored the mice's clinical parameters starting at 5 days post-infection (DPI), moreover, we performed cultivable fecal microbiota and gastrointestinal tract (GIT) function in the acute (10 DPI) and chronic (25 DPI) infection phases, when gut, lung and spleen were collected for histopathological analysis. KO mice exhibited worse clinical manifestations during infection, particularly during the acute phase when compared to IF-WT mice. We observed no difference in the number of brain cysts between the IF groups; however, the absence of eosinophils led to more inflammatory infiltrate in the alveolar parenchyma region and alteration in lymphoid organization in the spleen compared to the WT counterpart. The KO group showed increased GTI and anaerobic bacterial population at 10 dpi. At 25 DPI, no significant differences were observed in the gut between IF groups. Collectively, these findings demonstrate that eosinophils play an important role in tissue preservation and function during *T. gondii* experimental infection. (Funding: CNPq-CAPEs-FAPEMIG)

Keywords: Toxoplasmosis; Eosinophils; Immunoregulation.

2702 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

A new perspective on eosinophils: key mediators of parasitemia control, cellular activation and tissue damage during the severe malaria

SILVA, L.P.¹; RICCI, M.F.¹; SANTANA, L.F.D.¹; SANTOS, L.L.D.O.¹; OLIVEIRA, F.B.R.¹; TEIXEIRA, S.L.¹; DE FREITAS, G.M.¹; NONATO, A.¹; PEREIRA, R.D.D.¹; DE MEIRA, T.A.¹; MASSARDI, C.D.²; DE SOUZA, B.R.²; CASSALI, G.D.³; TEIXEIRA, M.M.¹; OLIVEIRA, N.F.D.M.¹; MACHADO, F.S.¹. 1. DEPARTAMENTO DE BIOQUÍMICA E IMUNOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS DA UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. DEPARTAMENTO DE FISIOLÓGIA E FARMACOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS DA UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. DEPARTAMENTO DE PATOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS DA UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Malaria is caused by Plasmodium, which the outcome depends on a fine balance of the immune system. Eosinophils contribute to regulation of immune cells and mediators. However, their role in severe malaria remains unclear. BALB-c (WT) and Δ dblGATA-1 (eosinophil-deficient, KO) mice were infected (IF) with 105 erythrocytes parasitized with Plasmodium berghei-ANKA. Clinical parameters, behavior tests, fecal microbiota, gastrointestinal motility, ELISA, cytometry, and histology were assessed at the setpoints. Eosinophil deficiency resulted in a drastic increase in parasitemia at 11dpi associated with the worst clinical score and susceptibility, leading to precocious mortality. KO mice presented an increase in circulating platelets and granulocytes at 12 dpi. Notably, KO-IF lung has higher inflammatory score, correlates with increased levels of IL6, IL17, IL10, TNF, and decreased levels of IL1 β , IFN- γ , and TGF- β compared to WT. Interestingly, the absence of eosinophils leads to higher gastrointestinal motility in the basal state, reversed at 12dpi when more damage in the KO-IF, but not in the WT-IF, colon was observed. KO showed a prevalence of anaerobic bacteria in the gut microbiota that remains at 12dpi. In addition, higher levels of Enterobacteriaceae, anaerobic lactic-acid bacteria, and lower in Staphylococcus at the 12dpi was observed in KO-IF than in WT-IF. The KO-IF brain presented an imbalance in key cytokines that may explain the changes in the motor and anxious behavior of these groups at the 12dpi. Finally, eosinophils modulate the generation-expansion of TCD4+ and TCD8+ cells in the spleen as well as their activation state. The increased expression of CTLA4+ rather than CD28+ in TCD4+ cells observed at the basal state, remains at 12dpi in KO-mice, which can explain, at least in part, the inability to control parasitemia. Our results suggest that eosinophils plays an important role against the malaria. (Funding: CNPq-CAPEs-FAPEMIG)

Keywords: Plasmodium; Immunoregulation; Microbiota.

2706 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Prediction of Immune Epitopes in ISC3, ISC6, and TSC5 Proteins of *Neospora caninum*: Potential for the Development of new diagnostic and vaccination strategies

DA CRUZ, L.F.M.; TORRES, J.D.A.; MINEO, T.W.P.. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL.

Neospora caninum is an obligate intracellular protozoan parasite of the phylum Apicomplexa, phylogenetically related to *Toxoplasma gondii*. It causes neosporosis, a disease associated with neuromuscular disorders in dogs and abortions in cattle, leading to significant economic losses in the livestock industry. This study aims to analyze and predict immunogenic epitopes from the ISC3, ISC6, and TSC5 proteins using the IEDB platform, focusing on both T lymphocyte responses (via MHC class I and II binding predictions) and B lymphocyte recognition (via B-cell receptor epitope prediction). Protein sequences were obtained from the ToxoDB database using the genomic data of the *N. caninum* reference strain, NcLiv. Epitope predictions for MHC class I and II were conducted for murine alleles, employing tools such as NetMHCpan and the Consensus method. Linear B-cell epitopes were predicted using the algorithm BepiPred. Selection criteria included high binding affinity, predicted immunogenicity, and cross-strain conservation. The *in silico* analysis revealed multiple epitopes with strong binding affinity to both MHC class I and II molecules, complementarily distributed across the three targeted proteins. Additionally, B-cell epitopes were identified in surface-exposed and accessible regions, suggesting their potential utility in vaccine formulations. These findings highlight ISC3, ISC6, and TSC5 as promising antigenic sources and potential candidates for the development of peptide-based diagnostic kits vaccines against bovine neosporosis. Furthermore, we are interested in performing gene knockout studies of these proteins to investigate their roles in parasite fitness, invasion, and egress, as well as to expand our understanding of their immunobiological relevance.

Keywords: Epitope prediction; *Neospora caninum*; In silico.

2713 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Circulating CD4⁺ T and B Cell Signatures as Potential Biomarkers of Cardiac Involvement in Chagas Disease

ANTONELLI, L.; DOS SANTOS, L.I.. FIOCRUZ, BELO HORIZONTE - MG - BRASIL.

Chronic Chagas cardiomyopathy (CCC), the most severe manifestation of *Trypanosoma cruzi* infection, is associated with distinct immune alterations. Using high-dimensional flow cytometry, we investigated the phenotypic landscape of circulating CD4^{pos} T and B cells in patients across clinical forms: asymptomatic (A), mild cardiomyopathy (B1), and moderate/severe cardiomyopathy (B2/C/D), compared to uninfected controls (CTL). We observed a marked expansion of effector CD4^{pos} T cells and effector memory (EM) subsets in CCC patients, particularly in B1. Mild CCC patients also exhibited higher frequencies of follicular helper T (Tfh) cells and transitional memory subsets expressing markers of activation (PD-1, ICOS, CD28, HLA-DR) and exhaustion (CD57, CD244). Conversely, central memory (CM) and stem cell-like memory (TSCM) subsets were reduced, especially in patients with severe forms of CCC. Unsupervised clustering confirmed these findings and revealed heterogeneous profiles of highly activated CD4^{pos} T cells correlating with cardiac involvement. In parallel, analysis of B cells revealed expansion of class-switched activated memory (AM) and atypical memory (AtyM) B cells in all infected groups, with the highest expression of activation markers (CD85j, TACI, CD32, CD40) in mild CCC. Naïve and transitional B cells were reduced in CCC, while class-switched IgA^{pos} and IgG^{pos} memory subsets predominated in B1 and B2/C/D patients. These findings suggest a sustained inflammatory response and altered immunoregulatory balance in patients with cardiac disease. Our data provide novel insights into immune signatures associated with different stages of CCC and support the use of T and B cell profiles as potential biomarkers of disease progression and immune-mediated tissue damage.

Keywords: Chagas disease; CD4^{pos} T cells; B cells.

2715 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Longitudinal Profiling of CD8 T Cell Subsets Highlights Effector Memory Expansion in Ocular Toxoplasmosis

DOS SANTOS, L.I.¹; RIMKUTE, I.²; LIECHTI, T.²; MARINO, A.P.³; HENRIQUES, P.M.³; SANTOS, D.V.⁴; MARTINS-FILHO, O.A.³; GAZZINELLI, R.T.³; ROEDERER, M.²; SHER, A.²; DE CARVALHO, A.T.³; JANKOVIC, D.²; ANTONELLI, L.³. 1. FIOCRUZ-MG/FACULDADE CIÊNCIAS MÉDICAS DE MG, BELO HORIZONTE - MG - BRASIL; 2. NATIONAL INSTITUTES OF HEALTH, BETHESDA - ESTADOS UNIDOS DA AMERICA; 3. FIOCRUZ-MG, BELO HORIZONTE - MG - BRASIL; 4. FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Toxoplasma gondii is a major cause of retinochoroiditis and uveitis in humans, yet the pathophysiological mechanisms underlying these manifestations remain poorly understood. Although CD8⁺ T cells play a key role in controlling *T. gondii* infection, little is known about their contribution to the clinical course of the disease or the kinetics of their subpopulations over time. Here, we analyzed samples from 21 patients with acute toxoplasmosis (AC), 6 months (6mo, n=21) and 12 months (12mo, n=25) after diagnosis. Based on ophthalmologic evaluation, individuals were stratified according to the presence (lesion) or absence (no lesion) of ocular involvement. High-dimensional flow cytometry was employed to investigate the phenotypic distribution and activation status of CD8⁺ T cell subsets in patients and uninfected controls (CTL, n=13). Our results demonstrated a significant expansion of activated CD8⁺ T cells (CD38⁺HLADR⁺) during the AC phase, which remained elevated at 6mo compared to CTL. This expansion was particularly evident in the effector memory CD8⁺ T cell subset, especially among individuals with ocular lesions. Conversely, the frequencies of central memory and stem cell-like memory CD8⁺ T cells were reduced during infection, with partial restoration observed over time. The expansion of effector memory CD8⁺ T cells in patients with ocular involvement at both acute and 6mo stages suggests a potential association between CD8⁺ T cell cytotoxicity and ocular pathology. These findings highlight distinct dynamics of CD8⁺ T cell subsets in *T. gondii*-infected individuals based on clinical stage and ocular status, offering insights into immune regulation and pointing to potential immunological targets for therapeutic interventions.

Keywords: human toxoplasmosis;CD8⁺ T cells;uveitis.

2722 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Modulation of Host Immunity by Trypanosoma cruzi Extracellular Vesicles via human Macrophages

TORRECILHAS, A.C.T.; NEGREIROS, N.G.S.; SARAIVA, N.. UNIFESP, SÃO PAULO - SP - BRASIL.

The protozoan *Trypanosoma cruzi* is the causative agent of Chagas disease and infects 6 to 7 million people worldwide. The protozoan parasite modulates host immune response, and the extracellular vesicles (EVs) released by *T. cruzi* trypomastigotes forms regulate intercellular communication and induce interactions with host cells. The aim of our study was to determine if the EVs released by *T. cruzi* can influence the immune response in human macrophages. Functional assays were performed to evaluate the interaction between EVs from *T. cruzi* and human macrophages. The EVs were labeled with PKH26 and incubated with macrophages (THP-1 cells differentiated with PMA). The effect of the interaction between *T. cruzi*-derived EVs and macrophages was analyzed using confocal microscopy and Amnis ImageStream cytometry at 1, 24, 48, and 72 hours. Cell viability was assessed by cytometry using 7-AAD and Annexin V staining, while cytokine levels in the cell culture supernatants were quantified by CBA. The results showed that EVs from *T. cruzi* interact with human macrophages and remain close to the nucleus after 72 hours of incubation (interaction). The EVs induce a cytokine production (TNF-alpha, IL-6, and IL-1) after 48 and 72 hours of incubation, and from 72 hours, we observed apoptosis in human macrophages. In Conclusions, the EVs released by *T. cruzi* interact with macrophages, modulate the host immune response, and induce apoptosis in the host macrophages. EVs can act as vehicles for the long-distance delivery of parasitic molecules, regulating macrophage functions. Macrophages are key cells during the early stages of *T. cruzi* infection and play a central role in the development of Chagas disease. Supported by FAPESP, CAPES and CNPq.

Keywords: extracellular vesicles;macrophage;Trypanosoma cruzi.

2726 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The impact of Extracellular Vesicles derived from *Trypanosoma cruzi* Trypomastigotes on Human Cardiomyocytes

GONÇALVES, M.O.; SARAIVA, N.; TORRECILHAS, A.C.T.. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Chagas disease, caused by *Trypanosoma cruzi*, represents a significant health concern in Latin America. Chronic Chagas cardiomyopathy, the most serious manifestation of the disease, affects approximately one-third of hospitalized patients. Several molecular invasion strategies are supported by parasite-released components, particularly extracellular vesicles (EV), which modulate immune responses and host tissue function. This study aims to clarify the interaction between *T. cruzi* EV and the host in association with cardiomyopathy. To evaluate the effect of EV on *T. cruzi* invasion, human cardiomyocyte cells (AC16) were subjected to different concentrations of EV prior to infection with *T. cruzi* trypomastigotes. Giemsa staining was used to quantify infected cells and intracellular parasites. Simultaneously, EV were labeled with PKH26 Dye Solution to visualize their interaction with AC16 cells. Cells were treated with stained EV at a 1:10 ratio for different times. Nuclei and trans-sialidase were also labeled. The essay was analyzed through Confocal Microscopy and Image Stream Cytometry. Our results demonstrate that higher EV levels enhance parasite invasion into host cells, suggesting a role for EV in *T. cruzi*-induced cardiac damage. Confocal images showed internalization of EV by AC16 cells in multiple locations, suggesting proximity to the nuclear membrane. Image Stream analysis confirmed EV translocation from the plasma membrane to the nuclear membrane, with co-localization of EV, trans-sialidase, and nuclei. Our data confirm the importance of EV in Chagas cardiomyopathy pathogenesis and parasite-host interactions by correlating elevated EV concentrations with enhanced parasite invasion. The co-localization of EV with trans-sialidase and their proximity to the nuclear membrane indicate a particular intracellular mechanism that may influence cellular activities and disease progression. Supported by FAPESP (2024/15997-5)(2020/07870-4), CNPq, and CAPES.

Keywords: Extracellular vesicles;Cardiomyocytes;Trypanosoma cruzi.

2727 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Development of a histopathological classification of lesions caused by *Leishmania (V) guyanensis* in patients with American Tegumentary Leishmaniasis: An immunohistochemical profile

DE SOUZA, L.V.O.¹; PEREZ, Y.G.²; DE MATOS, L.V.³; FARIAS, R.A.C.⁴; BARCELLOS, J.F.M.⁵. 1. FEDERAL UNIVERSITY OF AMAZONAS, MANAUS - AM - BRASIL; 2. ADRIANO JORGE HOSPITAL FOUNDATION, 69065-001, MANAUS, AM, BRAZIL, MANAUS - AM - BRASIL; 3. GRADUATE PROGRAM IN FRESHWATER BIOLOGY AND INLAND FISHING - NATIONAL INSTITUTE FOR AMAZONIAN RESEARCH - INPA - DOCTORATE, MANAUS - AM - BRASIL; 4. GRADUATE PROGRAM IN FRESHWATER BIOLOGY AND INLAND FISHING - NATIONAL INSTITUTE FOR AMAZONIAN RESEARCH - INPA - MASTER'S, MANAUS - AM - BRASIL; 5. DEPARTMENT OF MORPHOLOGY, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF AMAZONAS, MANAUS - AM - BRASIL.

Leishmaniasis is a disease considered a major public health problem in the state of Amazonas, caused by protozoa of the genus *Leishmania* with various clinically relevant manifestations and *Leishmania (V) guyanensis* is the most frequent in the Amazon region. Its clinical diagnosis depends on the identification of amastigote forms in skin samples, requiring several methods for confirmation. Thus, immunohistochemistry is used to obtain rapid diagnostic confirmation with high sensitivity. This study aims to validate the immunohistochemical method for diagnosing American Cutaneous Leishmaniasis based on the cross-reaction of antigens from species that cause the disease, with hyperimmune serum obtained from a naturally infected dog with *Leishmania infantum chagasi* in patients from the municipality of Rio Preto da Eva, Amazonas. Besides, 20 patients with a confirmed diagnosis who accepted the ethical participation term of the project were recruited. A skin fragment was then obtained through biopsy for subsequent histopathological and immunohistochemical analysis. *Leishmania* parasites were found in the skin and mucosa, more prevalent in men compared to women in the regions of Manopolis, Alto Rio Region, etc. Immunohistochemistry tests were positive in 8 women and 11 men, demonstrating *Leishmania* and parasitic antigens in the lesions of American Cutaneous Leishmaniasis were represented by amastigote forms and cellular marking, confirming infection in the main target cells of the parasites: dendritic cells and macrophages. Furthermore, lymphocytes were found in all lesions. The attempt at immunostaining with the antibody will still require adjustments. Subsequently, histopathology revealed in the lesions, granulomatous exudative reaction and cellular exudative reaction with the presence of tissue necrosis. In summary, this research will contribute to the validation of the Immunohistochemistry method as a strategic and effective diagnosis.

Keywords: Histopathology;Immunohistochemical;Amazon.

2730 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Modulation of Human Monocyte Differentiation by Extracellular Vesicles released by Trypomastigote Forms of *Trypanosoma cruzi*

IORIO, J.F.D.¹; PIETROBON, A.J.²; SATO, M.N.²; TORRECILHAS, A.C.T.¹. 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Trypanosoma cruzi is the protozoan responsible for Chagas disease, affecting 6–7 million people in Latin America. Diagnosis and treatment remain challenging. *T. cruzi* releases extracellular vesicles (EVs) that modulate inflammation via TLR2. Monocytes and macrophages are key players in immune responses, but the role of EVs in monocyte differentiation during early infection is not fully understood.

Objective: To evaluate the effects of EVs from trypomastigote forms of *T. cruzi* on the differentiation of human monocytes into macrophages.

Methods: Human monocytes were isolated from PBMCs by density gradient, Ficoll-Paque. For the invasion assays, monocytes were incubated with or without EVs at parasite concentrations 10^5 to 10^6 /mL. After incubation, infected cells and the number of intracellular parasites were evaluated. THP-1 cells treated with EVs (24 and 48h) were analyzed by SEM confocal microscopy, and flow cytometry (Amnis ImageStream, Leica DMI8). CD14, CD16, CD80, CD86, CD69, and HLA-DR markers were used.

Results: The results showed that *T. cruzi* EVs increase the number of intracellular parasites in cells and alter the morphology/differentiation of monocytes into macrophages. We detected the translocation of NF- κ B using confocal microscopy, as demonstrated by the increased endogenous expression of GFP, with NF- κ B pathway activation observed 30 minutes after the interaction; similar results were obtained by ImageStream. After 24 hours of incubation, we observed changes in the monocyte activation profile, including increased expression of CD14, CD16, CD80, CD86, CD69, and HLA-DR measured by flow cytometry

Conclusion: *T. cruzi* EVs interact with human monocytes, promoting activation, differentiation, and activation of the NF- κ B inflammatory pathway, impacting the innate immune response. Support: CAPES, CNPq, and FAPESP

Keywords: *Trypanosoma cruzi*; Extracellular vesicles; Monocytes.

2739 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Macrophages from subclinical individuals effectively control *Leishmania (V.) braziliensis* in phago-lysosomal compartments.

CARDOSO, T.M.D.S. FIOCRUZ-BA, SALVADOR - BA - BRASIL.

Introduction: Cutaneous leishmaniasis (CL) caused by *L. braziliensis* displays a wide spectrum of clinical manifestations, including subclinical infection (SC). SC produces less IFN- γ and TNF- α as compared to CL but controls infection. **Objective:** This study proposes experiments to elucidate the involvement of phagosome formation in the control of intracellular parasites in macrophages from SC individuals. The hypothesis is that SC macrophages present more effective phagosome/lysosome formation, activation, and duration. **Methodology:** Monocyte-derived macrophages were infected using calcein-labeled *Leishmania braziliensis* promastigotes to determine parasite death, CD107a expression, and cell activity markers by flow cytometry. **Results:** After 2 hours, the number of infected macrophages from SC, CL, and healthy subjects was similar, but after 48 hours, SC macrophages had fewer infected cells ($0.5 \pm 0.3\%$) compared to CL ($0.9 \pm 0.5\%$) and healthy controls ($2.7 \pm 1.8\%$). Additionally, SC had a higher frequency of macrophages CD107a+ ($52.8 \pm 22.5\%$) compared to cells from CL patients ($38.1 \pm 18.3\%$) after 48 hours of infection. SC macrophages also present a lower frequency of macrophages CD107a+ expressing iNOS ($1.5 \pm 2.5\%$) compared to CL macrophages ($3.0 \pm 1.7\%$) ($p < 0.05$). The frequency of infected macrophages from SC individuals expressing CD206 shows a positive correlation with the parasite load after 24 hours of infection, while infected cells with a CD107a+iNOS+ profile from CL patients showed iNOS expression with a positive correlation with the parasite load after 24 hours of infection. **Conclusion:** SC macrophages are able to form phagosomes faster, with lower iNOS production beside effective parasite control compared to CL macrophages.

Keywords: Subclinical infection; phagosome activity; leishmania killing.

2754 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Severe Malaria: Implications for a Regulatory Network of CD8⁺ T Cells and Tr1 CellsJACOBS, T.. BERNHARD-NOCHT-INSTITUTE FOR TROPICAL MEDICINE, HAMBURG - ALEMANHA.

In African children the leading cause of death is still. The individual susceptibility of children for malaria varies and the course can range from asymptomatic or uncomplicated infections to severe illness with life-threatening complications, including organ failure, severe anemia, or cerebral malaria. Neither host genetic factors nor parasitic factors alone can explain the course of illness found in malaria and in other infectious diseases as well. Recently, we have shown that an overwhelming immune response might contribute to severe malaria. Especially CD8⁺ T cells facilitate leakage of the brain endothelium ultimately leading to severe malaria. However, we found that these proinflammatory T cells can be regulated by type-I-regulatory T cells (Tr1 cells). Using high-content flow cytometry, we found strong induction of IL-10-producing Tr1 cells expressing various co-inhibitory molecules in acute malaria, which were capable to suppress T cell activation. By comparing children with uncomplicated or severe malaria, we found that Tr1 cells differ with regard to their expression of co-inhibitory molecules. In conclusion our data suggests that Tr1 cells dampen the overwhelming T cell response in severe malaria. However their function is modulated by expression of co-inhibitory molecules and a functional restriction of their regulatory capacity contributes to severity of disease.

Keywords: Malaria;T cells;Immune Regulation

2759 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Host-Pathogen Interaction: Inflammatory Responses of Human Macrophages to *Acanthamoeba polyphaga*PINTO, L.F.¹; ARAUJO, R.N.¹; POLITO, E.F.R.¹; FREIRE, A.S.¹; ALVES, J.L.B.¹; CRISTOVAM, P.C.¹; GATTI, Í.D.M.V.¹; DE FREITAS, D.¹; BORTOLUCI, K.R.². 1. UNIFESP, SÃO PAULO - SP - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL.

Some regions of the eye are immune-privileged, and the immune responses involved in *Acanthamoeba* keratitis (AK) remain poorly understood. This study aimed to investigate the inflammatory response of human macrophages challenged with *Acanthamoeba polyphaga* *in vitro*, with a focus on inflammasome activation. Macrophages derived from THP-1 monocytes and human monocyte-derived macrophages (hMDMs), pretreated or not with lipopolysaccharide (LPS; 200 ng/mL), were exposed to *A. polyphaga* (ATCC 30461) cysts and trophozoites at a multiplicity of infection (MOI) of 1:10 (one amoeba per ten macrophages) for 4, 24, and 48 hours. The infection rate, macrophage and amoeba viability, and the production of inflammatory mediators, such as nitric oxide (NO) and reactive oxygen species (ROS), were assessed. Inflammasome assembly was evaluated by quantifying ASC specks (apoptosis-associated speck-like protein containing a CARD), and IL-1 β secretion was measured. Both macrophage models, with or without LPS priming, internalized *A. polyphaga* cysts and trophozoites and significantly reduced amoeba numbers ($p < 0.0001$), indicating effective infection control. Moreover, infection-induced inflammasome assembly and IL-1 β secretion occurred despite low ROS production and absent NO release. A progressive decline in macrophage viability was observed over time, possibly reflecting cytotoxic effects mediated by inflammasome activation and/or the amoebae themselves. These findings suggest that human macrophages can internalize and control *A. polyphaga* infection while also triggering inflammasome activation, which may contribute both to the antimicrobial response and to macrophage loss during infection.

Keywords: Acanthamoeba keratitis;human macrophages;inflammasomes.

2775 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Immunometabolic reprogramming of macrophages by *Leishmania*: implications for parasite persistence

DA SILVA, G.D.¹; DE OLIVEIRA, E.C.¹; DOS SANTOS, R.T.T.²; LEAL, A.M.L.D.C.¹; MELO, L.D.³; ARAUJO, S.N.D.O.¹; DAVANZO, G.G.⁴; MARTINS, A.J.E.¹; RIBEIRO, B.V.¹; FRAGA, D.B.M.¹; DE MENEZES, J.P.B.¹; VERAS, P.S.T.¹; TAVARES, N.M.¹; VIEIRA, P.M.M.D.M.⁵; BRODSKY, C.I.¹. 1. FIOCRUZ BAHIA, SALVADOR - BA - BRASIL; 2. UNIVERSIDADE DA CALIFÓRNIA, SÃO FRANCISCO - ESTADOS UNIDOS DA AMERICA; 3. UNIVERSIDADE DE CALGARY, CALGARY - CANADA; 4. UNIVERSIDADE WASHINGTON, ST LOUIS, MISSOURI - ESTADOS UNIDOS DA AMERICA; 5. UNICAMP, CAMPINAS - SP - BRASIL.

Macrophages are key players in innate immunity, recognizing and eliminating pathogens. *Leishmania* spp. infection induces profound metabolic alterations in macrophages, increasing energy demands through mechanisms linked to mitochondrial integrity. However, how different *Leishmania* species modulate host metabolism remains unclear. This study investigates metabolic pathways critical for *Leishmania* persistence, focusing on parasite-driven host metabolic reprogramming. Bone marrow-derived macrophages (BMDMs) from C57BL/6 mice were infected with *Leishmania amazonensis* or *Leishmania braziliensis*. Metabolic activity was assessed using Seahorse analysis of extracellular acidification rate (ECAR) and oxygen consumption rate (OCR). Mitochondrial function was analyzed with MitoTracker probes, and parasite burden was quantified after treatment with metabolic inhibitors. Additionally, we examined parasite adhesion, phagocytosis, and mitochondrial reactive oxygen species (ROS) production using MitoSOX. Despite distinct immune evasion mechanisms, *L. amazonensis* and *L. braziliensis* induced similar metabolic shifts in macrophages. Infected cells exhibited increased glycolysis, elevated oxygen consumption, higher proton leak, increased mitochondrial mass and membrane potential, and reduced ATP production. Pharmacological inhibition of glycolysis and oxidative phosphorylation significantly reduced parasite burden, underscoring the dependence of intracellular survival on host metabolic pathways. Collectively, our findings reveal that despite species-specific immune evasion strategies, *L. amazonensis* and *L. braziliensis* similarly reprogram host metabolism to ensure intracellular survival. Both species consistently impact host bioenergetics, and pharmacological targeting of these pathways significantly reduces parasite survival, providing promising perspectives for therapeutic strategies against leishmaniasis.

Keywords: Immunometabolism; Macrophages; *Leishmania* spp.

2786 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Maturation, Phenotype, and Recruitment of Inflammatory Monocytes During Experimental Cerebral Malaria

RIBEIRO-ALMEIDA, M.L.; FREIRE-ANTUNES, L.; DE AZEVEDO, V.M.; ORNELLAS-GARCIA, U.; RANGEL-FERREIRA, M.V.; POEYS, A.C.D.S.; DE ASSIS, L.M.; DANIEL-RIBEIRO, C.T.; GOMES, F.L.R.. INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Malaria remains a major public health issue, with 263 million cases and 597 thousand deaths related to *Plasmodium* spp. infection in 2023. If not promptly diagnosed and treated, the disease can progress to severe forms, such as cerebral malaria (CM). Exacerbated immune activation has been recognized as a key factor in CM pathogenesis, with inflammatory monocytes (iMONs) considered potential drivers of disease progression. iMONs are produced in the bone marrow (BM), where their development and release into circulation are tightly regulated by CXCR4 expression. In the BM, a CXCR4^{hi} precursor population forms an immobilized pool that proliferates and replenishes the mature CXCR4^{low} population. This study aims to investigate the dynamics of iMONs production, migration, and polarization during *Plasmodium berghei* ANKA infection in C57BL/6 (ECM-susceptible) and BALB/c (ECM-resistant) mice. We analyzed BM, spleen, and brain at critical time points post-infection (days 4 and 6). Our findings reveal that C57BL/6 mice display a marked increase in BM iMONs, particularly the immature CXCR4^{hi} subset. While BALB/c mice also show an increase in CXCR4^{hi} iMONs in response to infection, the magnitude is lower and occurs in parallel with a decrease in mature CXCR4^{low} cells, suggesting a more controlled maturation and effective egress. In the spleen, BALB/c mice showed progressive iMONs accumulation, characterized by sustained CD62L expression and a gradual upregulation of the regulatory marker CD206. In contrast, C57BL/6 mice exhibited a transient splenic iMONs increase, displaying reduced CD62L and a higher IL-1 β expression. Despite distinct clinical outcomes, both strains recruited similar numbers of iMONs to the brain. These findings suggest that iMON phenotype, rather than presence alone, may be critical in CM development, highlighting their dual role in either exacerbating inflammation or promoting a better resolution depending on their activation state and microenvironmental.

Keywords: inflammatory monocytes; cerebral malaria; cxcr4.

2787 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Exploring Senescent and Inflammatory Features of Fibroblasts During Cutaneous Leishmaniasis

DOS SANTOS, I.M.F.; BORGES, T.M.; GOMES, D.C.D.O.; COVRE, L.P.. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL

Immunosenescence refers to a set of phenotypic and functional alterations in the immune system that arise during physiological aging or in response to environmental stressors such as chronic inflammation and persistent infections. These changes are typically associated with reduced immune responsiveness and the accumulation of dysfunctional cells. Senescent cells are characterized by limited proliferative capacity and the acquisition of a senescence-associated secretory phenotype (SASP), which contributes to chronic low-grade inflammation. Fibroblasts, key structural cells of the extracellular matrix, can undergo senescence and produce inflammatory mediators, thereby influencing the progression of chronic diseases. In cutaneous leishmaniasis (CL), patients develop ulcerative skin lesions marked by low parasite burden but intense inflammation and tissue damage. In this study, we aimed to investigate the presence of senescent and inflammatory fibroblasts within the lesions of individuals infected with *Leishmania braziliensis*. We evaluated canonical markers of senescence, including SA- β -gal, γ H2Ax, and p21, as well as SASP-related molecules such as IL-6, IL-8, and MCP-1. Our findings reveal the presence of senescent fibroblasts and heightened inflammatory signatures associated with these cells in CL lesions. These results suggest that fibroblasts may undergo phenotypic alterations during infection, potentially contributing to the inflammatory milieu observed in the disease.

Keywords: Fibroblastos ;Imunossenescência;Leishmaniose cutânea.

2790 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Inflammatory immune biomarkers are associated with disease progression and higher frequency of valvoplasties in rheumatic heart disease

RESENDE, A.L.D.S.¹; NEVES, E.G.A.²; MELO, L.D.S.¹; MOREIRA, T.B.²; JACAUNA, A.²; CARVALHO, A.T.³; NUNES, M.D.C.P.¹; DUTRA, W.². 1. FACULDADE DE MEDICINA DA UFMG, BELO HORIZONTE - MG - BRASIL; 2. UFMG, BELO HORIZONTE - MG - BRASIL; 3. 3INSTITUTO RENÉ RACHOU, FUNDAÇÃO OSWALDO CRUZ-FIOCRUZ, BELO HORIZONTE - MG - BRASIL.

Introduction: Rheumatic heart disease is a chronic complication of acute rheumatic fever caused by Group A *Streptococcus*. Its pathophysiology involves molecular mimicry between bacterial and cardiac antigens, triggering a dysregulated immune response. Activated T cells and autoantibodies target valvular tissue, leading to chronic inflammation and damage. In severe cases with stenosis, percutaneous mitral valvuloplasty (PMV) is often necessary. Identifying inflammatory biomarkers may enable early intervention to prevent irreversible cardiac injury.

Methods: we measured the plasma levels of immune molecules using the Bio-Plex pro human cytokine 48-plex kit from patients who underwent one PMV (n=68; 85% female) and those requiring ≥ 2 PMVs (n=22; 82% female).

Results: patients requiring ≥ 2 PMVs exhibited higher plasma levels of chemokines (CCL4, CCL5, CXCL9, CXCL12), the immune activation cytokine IL-2RA, the CD4⁺ T cell migration cytokine IL-16, and the fibrotic factor PDGF-BB than those with one procedure. These results are consistent with ongoing inflammation and fibrosis. Network analysis identified CCL5, CXCL10, CCL4, CXCL9, and IL-2RA as central hubs within the molecular interaction network, suggesting a pivotal role for these molecules in mediating inflammatory process in patients requiring multiple PMVs. Pathway enrichment analysis revealed involvement of the chemokine signaling pathway, Th1 and Th2 cell differentiation, Th17 cell differentiation, and TNF signaling pathway, highlighting key biological processes associated with increased expression of these molecules in patients with more severe disease.

Conclusion: systemic immune and inflammatory activation was higher in patients with two or more PMVs, who are considered clinically more severe. These biomarkers are associated with the severity of RHD and warrant further studies to evaluate their potential as targets to reducing valvular inflammation and fibrosis.

Keywords: Rheumatic Heart Disease;Inflammatory Biomarkers;Immunopathology.

2792 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

COMPLEMENT SYSTEM ACTIVATION IN PATIENTS WITH LEPTOSPIROSIS-ASSOCIATED PULMONARY HEMORRHAGIC SYNDROME

CARNEIRO, M.C.¹; DA SILVA, A.M.G.²; DA SILVA, L.F.F.¹; CRUZ, M.C.³; NETO, A.D.¹; ISAAC, L.¹. 1. SAO PAULO UNIVERSITY, SAO PAULO - SP - BRASIL; 2. INSTITUTE OF TROPICAL MEDICINE, SAO PAULO - SP - BRASIL; 3. CORE FACILITY TO SUPPORT RESEARCH, SAO PAULO - SP - BRASIL.

Leptospirosis is a neglected zoonotic disease with high mortality in severe cases, especially in patients who develop Leptospirosis-Associated Pulmonary Hemorrhagic Syndrome (LPHS). While the mechanisms underlying LPHS are not fully understood, they may involve leptospiral toxins that disrupt cell junctions and an exacerbated immune response leading to increased vascular permeability. The Complement System (CS) appears to play a role in LPHS pathogenesis, supported by C3 deposition and C3a receptor expression in affected lungs, though its precise contribution remains unclear. This study investigates CS activation in LPHS, the pathways involved, and its association with junctional disruption in response to pathogenic *Leptospira* infection. For that, post-mortem lung, kidney, and liver samples from LPHS patients (n=11) were compared to sepsis (n=4) and cardiopathy (n=6) controls. Immunohistochemistry was used to detect deposition of CS proteins (C1q, Factor B, MASP-2, C3c, C4d, C5b-9), anaphylatoxin receptors (C3aR1, C5aR1), immunoglobulins (IgG, IgM), and *Leptospira* antigens. The expression of junctional proteins (ZO-1, occludin, claudin-5/10, E-cadherin, and VE-cadherin) in lung tissue is currently being analyzed by immunohistochemistry. Following these analyses, the immunostaining quantifications will be compared with the histopathological evaluation of the tissue samples.

LPHS lungs showed more severe hemorrhage and inflammation than controls. Immunostaining revealed C1q deposition in endothelium, Factor B in alveolar fibrin and epithelium, and MASP-2 in vascular/epithelial regions, indicating all three CS pathways were activated. C1q and MASP-2 were significantly elevated, pointing to prominent Classical and Lectin Pathway involvement. Kidneys showed no significant CS activation. Liver and junctional analyses are ongoing. These findings support a role for CS in LPHS lung injury and suggest that CS inhibition may represent a promising therapeutic approach.

Keywords: Leptospirosis; Complement System; Pulmonary Hemorrhagic Syndrome.

2794 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The role of heme oxygenase-1 (HO-1) in *Cryptococcus neoformans* infection: from cellular response to systemic modulation

ALVES, T.P.; DUTRA, F.F.; GAMA, A.M.D.S.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Heme oxygenase-1 (HO-1) degrades free heme, generating anti-inflammatory byproducts that may protect the host during infections but can also induce immunosuppression. Its role in fungal infections remains poorly understood. *Cryptococcus neoformans* (Cn), a pathogen affecting immunocompromised individuals, initiates infection in the lungs and can disseminate to the central nervous system, causing severe meningoencephalitis. This pathogen survives inside macrophages, evading immune responses. Our study analyzed HO-1 expression and function in peritoneal macrophages from C57BL/6 mice during *in vitro* interaction with Cn (strain H99), as well as in the lungs and brains of intranasally infected mice. Macrophages were incubated with Cn for 16 hours *in vitro*, while mice were infected by intranasal instillation with 10⁵ fungal cells. HO-1 induction was assessed by Western blot and RT-qPCR. *In vivo*, HO-1 expression increased in the lungs at 7, 14, and 21 days post-infection. Daily treatments with CoPP (HO-1 inducer) and SnPP (HO-1 inhibitor) reduced fungal burden in the lungs on day 7 in both groups, but only CoPP maintained this reduction on day 14. In the brain, CoPP decreased fungal load on day 14. *In vitro*, HO-1 protein levels increased post-interaction (Western blot), but *Hmox-1* transcription (RT-qPCR) did not, suggesting that protein stabilization is critical for the cell accumulation of HO-1. ELISA and killing assays showed no significant cytokine production or fungal growth inhibition in our experimental condition. Thus, other protocols will be evaluated. Next steps include: (1) assessing whether CoPP/SnPP directly affect fungal biology; (2) investigating how CoPP prevents brain dissemination; (3) analyzing CoPP/SnPP effects on lung immune response; and (4) evaluating macrophage fungicidal activity in response to CoPP/SnPP. Understanding the role of HO-1 may advance cryptococcosis pathophysiology knowledge and support adjuvant therapeutic strategies.

Keywords: HO-1; *Cryptococcus neoformans*; cryptococcosis.

2814 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

NEW INSIGHTS INTO THE ANTIMICROBIAL ACTION FROM HTLV-1- INFECTED INDIVIDUALS TOWARD STAPHYLOCOCCUS AUREUS

ABREU, J.P.D.S.¹; DE OLIVEIRA, T.S.¹; VIANA, A.S.²; CARVALHO, M.F.¹; LEITE, A.C.C.B.³; LIMA, M.A.³; FIGUEIREDO, A.¹; OLSEN, P.C.¹; LIMA, J.E.N.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. BIOMANGUINHOS/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. INI/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Human T-cell lymphotropic virus type 1 (HTLV-1) infection is endemic in Brazil and is linked with HTLV-1-associated myelopathy (HAM). This chronic neuroinflammatory disease culminates in the loss of motor functions. HTLV-1 carriers exhibit a higher susceptibility to opportunistic infections, as caused by *S. aureus* (Sa). *S. aureus* secreted proteins were related to its virulence, as serine protease (Spl)D. This study aimed to investigate the anti-Sa immunoglobulins and antibody-mediated responses from HTLV-1-infected patients. **Methods and Results:** Sera were obtained from asymptomatic carriers, HAM, and uninfected individuals (NI). ELISA was used to evaluate the levels of total IgG, IgA, IgE, IgG anti-Sa (ST30, RN6390 and KB600-strain), IgA anti-Sa, IgG anti-SplD, and IgE anti-SplD. The HTLV-1-infected patients had higher levels of total IgG to NI but lower levels of IgE. Moreover, HAM patients showed higher serum levels of IgA. All groups exhibited higher levels of IgG anti-Sa RN6390 (full Spl operon) compared to IgG anti-KB600 strain (operon knockout Spl) and IgG anti-ST30 (SplD and A depleted). However, HAM patients presented lower levels of IgA anti-Sa than NI individuals. Specific levels of IgG anti-SplD were similar between groups, although the longitudinal analysis showed that this IgG level decreased. Then, we investigate the opsonophagocytosis capacity of monocytes using SYTO9 probe for Sa staining. Previously labeled bacteria were incubated with heat-inactivated serum, then opsonized bacteria were incubated with THP1 monocytes to evaluate phagocytosis by flow cytometry. Results showed that the opsonization promoted by serum from HTLV-1-infected patients induced lower phagocytosis than serum from NI individuals. **Conclusion:** The results suggested that HTLV-1-infected individuals have different serum conversion against *S. aureus*. This impacts the antibody-mediated responses and corroborates the higher susceptibility to infections of these patients.

Keywords: HTLV-1;S; aureus;Humoral immunity.

2815 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Study of Heme Oxygenase-1 Activity Modulation in Murine Macrophage Function and Its In Vivo Effects During Interaction with the Fungus *Sporothrix brasiliensis*

DA SILVA, J.B.D.P.; GAMA, A.M.D.S.; DE AMORIM, L.H.; NIMRICHTER, L.; DUTRA, F.F.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Sporothrix brasiliensis is one of the etiological agents of sporotrichosis. Heme oxygenase-1 (HO-1) is an isoenzyme involved in heme catabolism, generating products with anti-inflammatory effects. Thus, our hypothesis is that HO-1 increases host susceptibility by reducing host resistance. Our aim was to investigate the role of HO-1 in the interaction between *S. brasiliensis* (MYA-4823) and peritoneal macrophages (PMs) from C57BL/6 mice, as well as in a subcutaneous murine model of sporotrichosis. RT-qPCR and western blot analysis indicated increased expression and accumulation of HO-1 in infected skin. The murine infection was monitored for 35 days: mice treated with cobalt protoporphyrin IX (CoPP), an HO-1 inducer, died within 21 days and exhibited weight loss, in contrast to untreated animals, which survived and gained weight. Moreover, after 15 days, CoPP treatment resulted in increased spleen weight and higher CFU counts in the skin and spleen, suggesting reduced host resistance. Conversely, treatment with tin protoporphyrin IX (SnPP), an HO-1 inhibitor, led to 40% mortality despite weight gain, indicating a distinct effect compared to the other treatment groups. Additionally, in the SnPP-treated group, skin lesion healing was delayed, and by day 35, splenomegaly and hepatic nodules were observed, indicating enhanced fungal dissemination. However, at 15 days, SnPP did not alter spleen weight or CFU counts, suggesting its effects occur at later stages of infection. In vitro, PMs incubated for 24 hours with conidia or yeasts induced increased HO-1 expression, as detected by western blot. Regarding the microbicidal capacity of PMs, incubation with opsonized conidia did not reduce viable fungal counts; however, PMs treated with SnPP exhibited enhanced fungicidal activity. Activation of PMs with IFN- γ plus LPS promoted conidial elimination, and this effect was reversed by CoPP treatment. Further experiments are needed to clarify the role of HO-1.

Keywords: HO-1;Sporothrix;Macrophage.

2816 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Urinary extracellular vesicles and inflammatory mediators in the post-COVID condition

DE OLIVEIRA, E.M.¹; NASCIMENTO, C.D.M.C.¹; DO ROSÁRIO, N.F.¹; RODRIGUES, C.¹; DA SILVA, I.B.¹; RAMOS, A.¹; CARVALHO, F.R.¹; BARBOSA, P.²; DE FELICE, F.G.²; CASTRO, M.J.C.¹; LUGON, J.R.¹; AVELAR, T.M.T.¹; SILVA, A.A.¹. 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL; 2. D'OR INSTITUTE FOR RESEARCH AND EDUCATION, RIO DE JANEIRO - RJ - BRASIL.

Introduction: The post-COVID condition (PCC) was defined by the World Health Organization as the persistence or emergence of symptoms up to two months after SARS-CoV-2 infection that persists for at least three months. This study investigated the association between circulating and urinary inflammatory mediators, extracellular vesicles (EVs), and clinical and laboratory findings during the post-COVID-19 period. **Methods:** This is a cross-sectional study conducted with individuals with history of COVID-19 stratified according to PCC and hospitalization during the acute phase. Multiplex assays quantified circulating and urinary levels of 27 inflammatory mediators. EVs were isolated by differential centrifugation and assessed by nanoscale flow cytometry, nanoparticle tracking analysis (NTA), and transmission electron microscopy. **Results:** We included 78 participants (55±14.6 years-old; 79.5% females), of whom 56 (71.8%) had PCC. Of these, 18 (32%) required hospitalization during COVID-19. No differences between groups were observed regarding plasma EVs, but hospitalized PCC patients presented lower levels of circulant IL-9 (p=0.03) and laboratory alterations such as higher monocyte-to-lymphocyte ratio (p=0.03), prothrombin time (p=0.02), and lactate dehydrogenase (p=0.01). In addition, higher levels of total urinary EVs (p=0.006) and urinary IL-4 (p=0.01), CCL-2 (p=0.02), CCL-11 (p=0.002), and GM-CSF (p=0.04) were observed in the same group. Likewise, individuals infected before vaccination presented with a higher total uEVs (p=0.003) count and urinary CCL-11 (p=0.01) levels, and multiple episodes of COVID-19 were associated with higher urinary IFN γ (p=0.04) and IL-1Ra (p=0.03) levels. **Conclusion:** Our results suggest a possible remnant renal inflammatory process in PCC patients who had moderate-to-severe acute COVID-19.

Keywords: post-COVID-19 condition;extracellular vesicles;inflammation.

2818 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Comparison of T Cell and Antibody Responses Between Convalescent and Non-Convalescent COVID-19 Vaccinees

DACRI, I.M.; RODRIGUES, D.A.S.; GABRIELLE, B.; VALE, A.M.; CASTIÑEIRAS, T.M.P.P.; OTA, V.A.; DE LIRA, G.S.; BOZZA, M.T.; LIMA, J.E.N.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

COVID-19 is a respiratory infection caused by the SARS-CoV-2 virus, which was officially declared a global pandemic in March 2020. Brazil began its COVID-19 immunization program in February 2021, which significantly reduced the number of deaths and severe cases. The vaccine promotes a robust humoral and cellular immune response, generating a specific memory. Activated CD4⁺ T lymphocytes can differentiate into T follicular helper cells (T_{fh}), which assist B lymphocytes in immunoglobulin production and class switching. Individuals recovered from COVID-19 exhibit memory B and T cell responses as well as the production of serum neutralizing IgA and IgG. However, the emergence of new viral variants led to cases of reinfections even in vaccinated individuals. This study hypothesizes that a previous infection with SARS-CoV-2 modulates T cell and antibody responses induced by vaccines. Volunteers from Núcleo de Enfrentamento e Estudos de Doenças Infecciosas Emergentes e Reemergentes/UFRJ vaccinated with 3 doses of CoronaVac, AstraZeneca, or Pfizer vaccine (2021) were stratified into 3 groups: uninfected before the 1st dose, infected before the 1st dose, and infected after the 1st dose or between the 2nd and 3rd doses. In our results, we observed that previous or subsequent infection did not influence the frequency of Spike-specific CD4 and CD8 T cells expressing different activation and memory markers after 3-7 days of 3rd dose. However, individuals infected before the first dose showed higher CD69 expression in circulating T_{fh} cells compared to individuals not previously infected after specific cell stimulation. Furthermore, individuals infected between the three doses had higher serum specific IgA and IgG titers (anti-Spike ancestral and omicron) compared to uninfected individuals before the three doses. We concluded that an infection before vaccination subtly alters circulating T_{fh} cells but positively modifies serum-specific IgA and IgG titers.

Keywords: COVID-19;Immunoglobulin;Vaccine.

2820 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Host handling of parasite-derived debris in cystic echinococcosis: analysis of Clec4F-independent mechanisms

BARRIOS, A.; CASARAVILLA, C.; OLEGGINI, A.; DÍAZ, Á.. UNIVERSIDAD DE LA REPÚBLICA, MONTEVIDEO - URUGUAI.

Cystic echinococcosis is caused by the larval stage (hydatid) of *Echinococcus granulosus* s.l. Hydatids are bladder-like structures that grow within internal organs of ungulates and humans. They are surrounded by a thick acellular coat called laminated layer (LL), composed of mucins and calcium inositol hexakisphosphate (InsP₆) nanodeposits. During infection, the LL is opsonized with anti-mucin antibodies. Parasite growth requires LL shedding. Host handling of this debris may impact parasite immune responses. In experimental mouse infections, we found that LL materials (LLm) circulate and are selectively captured by liver Kupffer cells (KC), mainly via the C-type lectin Clec4F (exclusively expressed by KC in rodents). Similar results were obtained after injection of LL mucin preparations. Since humans lack functional Clec4F, we are now exploring Clec4F-independent uptake mechanisms, suggested by the previous results in mice. Such mechanisms could include opsonization by complement and/or specific antibodies. In Clec4F deficient (*Clec4f*^{-/-}) mice, LL clearance from circulation was slower than in wild-type (WT) mice but LLm were eventually cleared, confirming the existence of Clec4F-independent uptake mechanisms. In the absence of both Clec4F and specific antibodies, injected LLm were taken up by unidentified cell types in spleen and lymph nodes, KC, and by peritoneal macrophages. InsP₆ is known to bind C1q, initiating limited antibody-independent activation of the classical pathway (CP). However, this mode of CP activation did not affect LLm uptake by cultured peritoneal macrophages (CPM) or by KC or peritoneal macrophages *in vivo*. Preliminarily, we also observed some alternative pathway (AP)-mediated uptake by CPM. In contrast, in the presence of specific antibodies there was strong C1q-dependent uptake of LL by CPM, suggesting CP involvement. We are currently assessing possible roles of the AP and of antibodies in the uptake of LLm by different cell types *in vivo*.

Keywords: *Echinococcus granulosus*; LL uptake; complement/antibodies.

2828 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Lack of Hypoxia-Inducible Factor-1 alpha (HIF-1α) Influences on Macrophages Ability to Deal with Leishmania braziliensis In Vitro and Affects Pathology In Vivo

MARINHO, F.A.V.¹; SANCHES, R.C.D.O.¹; VAZ, L.G.¹; GUIMARÃES, E.S.¹; DE CARVALHO FILHO, E.M.²; DE CARVALHO, L.P.²; DE OLIVEIRA, G.C.¹; OLIVEIRA, S.C.³. 1. FEDERAL UNIVERSITY OF MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL; 2. FEDERAL UNIVERSITY OF BAHIA (UFBA), SALVADOR - BA - BRASIL; 3. UNIVERSITY OF SÃO PAULO (USP), SÃO PAULO - SP - BRASIL.

Cutaneous leishmaniasis, caused by *Leishmania braziliensis*, still represents a serious health problem in Brazil, especially in the northeast region. Currently, to our knowledge, no report describes the role of hypoxia-inducible factor-1α (HIF-1α) during *L. braziliensis* infection. In this study, we demonstrated that the parasite induces HIF-1α expression and stabilization in bone marrow-derived macrophages only when added with exogenous IFN-γ plus lipopolysaccharide. Coherently, we did not find an enrichment in the glycolytic pathway upon bone marrow-derived macrophage infection. Evaluating the impact of HIF-1α absence during macrophage infection *in vitro*, we observed HIF-1α-knockout cells present high levels of IL-10, reduced production of nitric oxide, and decreased expression of VEGF-A. As a result, parasite viability improves within HIF-1α-knockout cells. However, *in vivo*, the absence of myeloid cells expressing HIF-1α had no influence on nitric oxide levels at tissue and in parasite burden. Conversely, lack of HIF-1α significantly affects *L. braziliensis*-induced pathology. Ear lesions induced in myeloid HIF-1α-knockout mice were thicker, presenting higher frequency of macrophages, neutrophils, CD4⁺, and CD8⁺ T cells, as well as higher levels of IL-12, IL-1β, and IFN-γ, compared with those in wild-type mice. Moreover, draining lymph nodes from myeloid HIF-1α-knockout mice also harbored increased populations of T cells. Our data demonstrate that HIF-1α plays an important role during *L. braziliensis* infection influencing skin pathology *in vivo*.

Keywords: HIF-1α; Innate immunity; *Leishmania braziliensis*.

2834 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

DHA-RICH OMEGA-3 SUPPLEMENTATION PRIOR TO *Plasmodium berghei* NK65 INFECTION DOES NOT ALTER LUNG INJURY IN MICE

MACHADO, L.F.¹; PEREIRA, L.P.M.C.¹; RODRIGUES, J.H.S.¹; PINHEIRO, F.M.¹; SILVA, A.C.F.¹; MARQUES, C.B.¹; GOMES, M.T.¹; ROCHA, V.N.¹; NETO, A.F.¹; GOMES, F.L.R.²; CASTRO, J.M.D.A.¹; SCOPEL, K.K.G.¹. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Acute Respiratory Distress Syndrome (ARDS) is a severe and often fatal complication of malaria, resulting, among other factors, from an exacerbated inflammatory response induced by infection. Interestingly, studies have shown that ARDS can occur independently of infective parasite strain; however, no effective treatment is currently available for this condition. Our group previously demonstrated that DHA-rich fish oil supplementation reduced lung inflammation and disease severity in mice infected with *Plasmodium berghei* ANKA, a strain that induces exacerbated inflammation with parasite and leukocyte adhesion to endothelial tissue. However, it remains unclear whether omega-3 supplementation with high DHA doses is effective against pulmonary injury from other *Plasmodium* species, such as *P. berghei* NK65, which triggers systemic inflammation but not endothelial adhesion. Given the lack of specific treatments for malaria-associated ARDS and the distinct pathogenic profiles between strains, this study investigated whether DHA-rich fish oil could modulate pulmonary injury caused by PbN infection in a murine model. Female C57BL/6 mice were supplemented orally with 3 g/kg of DHA-rich omega-3 for 15 days before infection. Pulmonary injury was evaluated through measurement of lung oedema ratio, alveolar-capillary permeability assays, and histological analysis. The inflammatory environment was assessed through IFN- γ /IL-10 ratio in lung homogenates. Additionally, total and differential cell counts, hydrogen peroxide production, and protein concentration were quantified in bronchoalveolar lavage fluid to evaluate inflammation and oxidative stress. Pulmonary immune cell phenotypes were assessed through flow cytometry. Nonetheless, no statistically significant differences were observed between DHA-supplemented and control-infected groups. These results indicate that DHA-rich fish oil supplementation does not modulate lung injury or pulmonary inflammation in this experimental model.

Keywords: severe malaria;lung injury;omega-3 PUFAs.

2849 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Modulation of PD-L1 Expression in Neutrophils Following *Plasmodium berghei* ANKA Infection and Secondary Inflammatory Challenge

DE AZEVEDO, V.M.; RIBEIRO-ALMEIDA, M.L.; POEYS, A.C.D.S.; BAPTISTA, B.J.; DE ASSIS, L.M.; ORNELLAS-GARCIA, U.; FREIRE-ANTUNES, L.; DANIEL-RIBEIRO, C.T.; GOMES, F.L.R.. FIOCRUZ/RJ, RIO DE JANEIRO - RJ - BRASIL.

Malaria is a disease caused by protozoan parasites of the *Plasmodium* genus, responsible for thousands of deaths annually. While the innate immune system limits parasite growth, an unbalanced inflammatory response contributes to severe manifestations, such as cerebral malaria (CM). Neutrophils, key players in the host defense, produce inflammatory mediators that amplify inflammation and shape adaptive immunity. Recent studies have revealed phenotypic and functional diversity among neutrophil. Moreover, innate immune cells are now understood to exhibit a form of memory, whereby previous exposures to infections or vaccines can modulate their responses, upon encountering subsequent stimuli. This study aimed to assess whether *P. berghei* ANKA infection in C57BL/6 mice, a model for CM, induces immune memory in neutrophils. Mice were infected and treated with chloroquine before CM signs emerged. After recovery, mice were challenged with lipopolysaccharide (LPS) to evaluate whether activation or suppression markers persisted in neutrophils, potentially indicating the establishment of innate immune memory. Our data revealed that, during active infection, the expression of PD-L1, a co-inhibitory molecule associated with immune regulation, increased significantly in splenic and bone marrow-derived neutrophils. Following treatment, at a later stage, neutrophils from previously infected animals exhibited a considerable reduction in PD-L1 expression. However, upon secondary LPS challenge, PD-L1 levels increased again but remained significantly lower than those in LPS-challenged, non-infected controls. This modulation was observed in both spleen and bone marrow compartments. These findings suggest that prior plasmodial infection altered the capacity of neutrophils to respond to inflammatory stimuli. While reduced PD-L1 expression may reflect altered regulatory potential, further investigation is needed to determine whether this represents the development of innate immune memory.

Keywords: Malaria;Neutrophils;Innate immune memory.

2850 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

First report of sensitization to house dust mite *Glycycometus malaysiensis* in Brazil: A new player in mite-induced allergy

DE SOUZA, L.M.¹; SENA, N.C.²; DA SILVA, E.S.³; SOUSA, J.E.A.³; DE ANDRADE, J.C.D.C.³; SANTOS, M.L.³; PACHECO, L.G.C.³; PINHEIRO, C.D.S.³. 1. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. UFBA, SÃO SEBASTIÃO DO PÁSSÉ - BA - BRASIL; 3. UFBA, SALVADOR - BA - BRASIL.

House dust mites (HDMs) are recognized as one of the most important sources of indoor allergens worldwide, which can develop immunoglobulin E production. They are strongly associated with the development of allergic diseases such as asthma, allergic rhinitis, and atopic dermatitis. Among these, *Glycycometus malaysiensis* is a mite species found in house dust, which is morphologically similar to *Blomia tropicalis*, and for the first time in Brazil, the storage mite *G. malaysiensis* was found in house dust mites in 70% of the samples. Although its allergenic potential is mentioned in the literature, there are few research results related to *G. malaysiensis* and the molecular characterization of allergens. **Objectives:** Produce an allergenic extract and evaluate its reactivity using serum from atopic and non-atopic individuals through ELISA. Additionally, to analyze the mite's proteins using one-dimensional electrophoresis and immunoblotting. **Material and Methods:** Dust samples were obtained from homes in the city of Salvador, Bahia, and identified through PCR-based techniques. Protein extracts were then obtained from pure cultures of *Blomia tropicalis* and *Glycycometus malaysiensis*. These protein extracts were utilized in indirect ELISA assays and immunoblotting to evaluate IgE reactivity, using sera from atopic and non-atopic individuals from Salvador, Brazil and Colombian. **Results and conclusions:** The analysis in sera from Salvador-Bahia revealed that atopic patients exhibited IgE reactivity to the extracts containing *B. tropicalis*, *D. pteronyssinus* and *G. malaysiensis*. Interestingly, 4 individuals demonstrated IgE reactivity only for *G. malaysiensis*. Conversely, the sera from Colombian individuals, IgE reactivity was detected against *B. tropicalis* and *D. pteronyssinus*, however, no reactivity was observed for *G. malaysiensis*. This study underscores the immunogenic potential of *G. malaysiensis* and its significance within the tested population from Salvador, Brazil. **Keywords:** House dust mite; allergies; *G. malaysiensis*.

2868 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Senescent-like Cytotoxic CD4+ T cells could target CD68+ and Vimentin+ cells contributing to lesional tissue damage during Localised Cutaneous Leishmaniasis

DE QUEIROZ, A.M.¹; LOPES, P.D.O.¹; DE CASTRO, C.H.D.F.¹; COVRE, L.P.¹; DE CARVALHO, L.P.²; FALQUETO, A.¹; AKBAR, A.³; MOSSER, D.⁴; GOMES, D.C.D.O.¹. 1. FEDERAL UNIVERSITY OF ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 2. FIOCRUZ-BA, SALVADOR - BA - BRASIL; 3. UNIVERSITY COLLEGE LONDON, LONDON - INGLATERRA; 4. UNIVERSITY OF MARYLAND, COLLEGE PARK - ESTADOS UNIDOS DA AMÉRICA.

Localised Cutaneous Leishmaniasis (LCL) is a severe skin infectious disease caused by Leishmania parasites. The immune response is crucial in the pathogenesis of this disease, which is characterised by destructive lesions and chronic inflammation. Chronic stimulatory processes and infections can guide the establishment of cell senescence phenotype. Senescent immune cells can present functional disorders as well as intense inflammatory activity. One of the distinct features of these cells is the acquisition of natural killer cell receptors (NKR) in T cells and a robust, non-specific cytotoxic response. In this work, using transcriptomic analysis and multiplex immunofluorescence, we evaluate the presence of CD4+ T cells with senescence features and its cytotoxicity through NKG2D, NKG2C and Granzyme B expression. Furthermore, we assessed the expression of NKR's ligands and the spatial proximity of CD68+ and Vimentin+ cells to cytotoxic CD4 lymphocytes. Our analyses demonstrate that LCL patients exhibit remarkably high senescence and cytotoxicity scores, with a strong correlation between them. Together with these signatures, we demonstrate that CD4+ T cells express senescence markers and that cytotoxic CD4+ T cells accumulate in the skin, especially CD4+GZMB+NKG2D+ cells (CD4 CTLs). In addition, we demonstrate that CD68+ and Vimentin+ cells express the NKG2D ligand, MICAB, and are distributed in the tissue and localised within 10 µm distance of CD4 CTLs. Finally, we demonstrate that the accumulation of these cells only occurs in LCL and not in the diffuse cutaneous (DCL) form of leishmaniasis, a less pro-inflammatory disease manifestation without ulcerated lesions. Taken together, these results suggest that the senescent-like CD4 CTLs population could contribute to aggravation of the damage, and consequently inflammation, observed in lesions of LCL patients.

Keywords: Leishmaniasis; Immunosenescence; Cytotoxic CD4+ T cells.

2874 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

INFLAMMATORY RESPONSE TO *Corynebacterium ulcerans* IN A HUMAN AIRWAY CO-CULTURE MODEL UNDER AIR-LIQUID INTERFACE CONDITIONS

ANDRADE-SILVA, M.¹; BOTELHO, M.A.²; LIMA, M.V.D.S.²; SOUSA, A.M.²; DURAO, C.V.²; DE OLIVEIRA, E.E.²; SILVA, F.M.D.C.E.²; FRANÇA, F.L.D.S.²; OLIVELLA, J.G.B.²; SANT'ANNA, L.D.O.²; SANT'ANNA, L.S.D.S.²; DE MATTOS-GUARALDI, A.L.². 1. FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. RIO DE JANEIRO STATE UNIVERSITY, RIO DE JANEIRO - RJ - BRASIL.

Background: One Health is the concept that human, animal, and environmental health are closely interconnected and interdependent, and that collaborative efforts across these sectors are essential to achieving optimal health outcomes. *Corynebacterium ulcerans* exemplifies this concept, as its ecological relationship with animals and humans is directly related to its pathogenic potential. In this context, the present study aimed to apply a co-culture model combined with the air-liquid interface (ALI) technique, to investigate host-pathogen interactions. **Methods:** A549 cells (human alveolar epithelial cells, ATCC CCL-185) were cultured in Transwell inserts (0.4 µm) for 13 days under air-liquid interface (ALI) and liquid-liquid interface (LLI) conditions. The cells were then maintained for an additional day in indirect co-culture with macrophage-like cells (MØ-like) derived from THP-1 monocytes (ATCC TIB-202). After one day of co-culture, the cells were challenged with *C. ulcerans* 809 (isolated from a fatal case of human pneumonia) under three conditions: apical (infection of the A549 cells only), basolateral (infection of MØ-like only), and both sites simultaneously. Cytokine levels (interleukin [IL]-6, IL-10, IL-1β, and tumor necrosis factor-alpha [TNF-α]) were measured by enzyme-linked immunosorbent assay (ELISA) after 20 hours of challenge. **Results:** Basal IL-6 levels were significantly higher in co-cultures of A549 cells (ALI and LLI) with MΦ-like cells compared to monocultures. No basal differences were observed for IL-1β or TNF-α. Upon infection, TNF-α levels decreased and IL-1β levels increased when MΦ-like cells or both cell types were infected, compared to co-culture control and both monocultures. IL-6 levels increased when only MΦ-like cells were infected, but not when both cell types were infected. **Conclusion:** In this sense, the co-culture model seems to significantly influence cytokine levels through soluble factors in infections with *C. ulcerans* 809.

Keywords: *Corynebacterium ulcerans*;One health;Cytokine.

2885 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The role of eosinophils during *Cryptococcus gattii* infection

FERREIRA, E.G.; DA SILVA JUNIOR, E.B.; FREIRE-DE-LIMA, C.G.; DE MELO, C.B.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Cryptococcosis is a potentially systemic invasive mycosis responsible for approximately 625,000 deaths annually. Species from the *Cryptococcus neoformans*/*C. gattii* complex are the primary etiological agents of the disease, with lung tissue generally serving as the primary site of infection. Eosinophils are prominent cells in bronchopulmonary mycoses. However, the exact role of eosinophils during fungal infections, particularly in pulmonary cryptococcosis, remains poorly understood. Previous work from our group has shown that pulmonary infection with *C. gattii* is associated with a type 2 inflammatory response, characterized by increased eosinophils, elevated IL-4 and IL-5 levels, and significant fibrosis caused by the deposition of thick collagen fibers. We hypothesize that eosinophils play a central role in the pulmonary inflammatory response caused by *C. gattii*. To investigate the contribution of this leukocyte population during *C. gattii* infection, we intratracheally inoculated 10⁴ yeasts into BALB/c wild-type (WT) and eosinophil-deficient (Δ dblGATA1^{-/-}) mice. Our results indicate that Δ dblGATA1^{-/-} mice are more susceptible to *C. gattii*, displaying higher mortality rate compared to infected BALB/c WT animals. While infected BALB/c WT mice show intense lung eosinophilic infiltration both infected mice displayed similar overall leukocyte alterations. Additionally, Δ dblGATA1^{-/-} mice exhibit higher clinical score two weeks post-infection with apparent fungal dissemination to the brain. Further data are still needed to address our hypothesis, but the present results suggest a protective role for eosinophils during pulmonary *C. gattii* infection in the BALB/c mice model.

Keywords: cryptococcosis;eosinophils;inflammation.

2890 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Pirahy virus: characterization of a new and potential emerging arbovirus

HUBNER, L.J.. UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRASIL.

One way to prevent the damage caused by the emergence of new diseases is through surveillance and investigation, which enables the discovery of new pathogens. Pirahy (PIRAV) was an alphavirus identified in southern Brazil in 2022 in mosquitoes of the genera *Trichoprosopon* and *Wyeomyia*. Genomic analyses classified PIRAV within the Venezuelan equine encephalitis virus (VEEV) complex, and studies in cells showed that it is capable of infecting human cells. These factors classify PIRAV as a potential virus capable of causing disease in humans, and therefore, in this study, we investigate risk parameters associated with its emergence. First, we evaluate whether PIRAV can be transmitted by *Aedes aegypti*, which is already a vector of several diseases such as Zika and Dengue. We infected the mosquitoes with 10^7 PFU/ml of the virus through blood feeding. At 7 days post-infection (d.p.i), we obtained 100% infection prevalence, which reduced to 75% by 21 d.p.i. At 14 d.p.i., we collected the saliva of the mosquitoes and found 3/34 infected. These data confirm that PIRAV can be transmitted by *A. aegypti*, although still inefficiently. To understand how this virus activates the Interferon pathway, we infected A549 WT and RIG-I ko cells. The viral titer was lower in WT cells, which can inhibit infection within 72 hours, unlike RIG-I ko cells, where virus replication only increases over time. Although, cell viability was lower in WT cells, indicating a failure in the activation of immunity. This hypothesis is reinforced by qPCR results in which we observed the induction of IFNB, IFNL1, IFIT2, and NFKBIA expression in WT but not in RIG-I ko cells. This indicates that RIG-I is the main sensor that recognizes PIRAV and leads to IFN activation. Through these results, we show that PIRAV is a good target for studies on emerging diseases, and that evolution and adaptation processes can favor both its transmission by *A. aegypti* and its infection in human cells.

Keywords: Arboviruses;Emerging viruses;Interferon.

2892 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The inhibition of TAM RECEPTOR AXL induces a M2 to M1 shift and helps macrophages to control *Trypanosoma cruzi* infection

DA SILVA, T.D.C.M.; BARROSO, N.V.G.; LOPES, M.D.F.. INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO - UFRJ, RIO DE JANEIRO - RJ - BRASIL.

In Chagas disease, caused by *Trypanosoma cruzi*, the immune system controls acute infection, but fails to prevent chronic infection and heart pathology. T cells eliminate infected cells and produce IFN- γ , which activates M1 macrophages to eliminate the parasite. However, T cells undergo apoptosis and are phagocytosed (efferocytosis) by macrophages inducing parasite-permissive M2 macrophages. The TAM receptors Axl and MerTK are involved in efferocytosis and inhibitory signaling in macrophages. Previous studies with Axl receptor-sufficient and deficient mice showed that Axl induces efferocytosis and reduces M1 responses in vitro and increases parasitemia and heart pathology during infection. Thus, we investigated how pharmacological inhibition of Axl-mediated efferocytosis impacts macrophage-mediated immunity against *T. cruzi*.

We first evaluated the effect of the Axl inhibitor R428 on efferocytosis. Bone marrow-derived macrophages (BMDMs) from C57BL/6 (B6) mice were co-cultured for 48 h with CFSE⁺ apoptotic thymocytes and R428 at concentrations of 10, 20, and 30 nM, which are not toxic to macrophages. Flow cytometry showed a dose-dependent reduction in apoptotic cell uptake. In 48 h co-cultures of T cells from infected mice (17 dpi) with WT BMDMs, R428 reduced MGL-1 and Arg-1 expression (M2 markers), increased induced NO synthase expression (M1 marker), and increased NO production in a dose-dependent manner. These effects were absent in Axl^{-/-} macrophages, indicating the specificity of the Axl inhibitor. In 72 h co-cultures with *T. cruzi*-infected macrophages, optical microscopy revealed a reduced parasite load at 20 and 30 nM of R428. R428 had no direct effect on parasite load in macrophages cultured without T cells.

The Axl inhibitor R428 modulates efferocytosis in vitro, inducing M1 macrophages with higher microbicidal activity and reduced parasite burden, while reducing the infection-prone M2 phenotype. Thus, Axl is a potential therapeutic target for Chagas disease.

Keywords: *Trypanosoma cruzi*;M1/M2 Macrophages;The Axl inhibitor R428

2903 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Exosomes, microRNAs, and immune signatures as predictors of dengue severity: a pilot study in hospitalized young adults

AGUDELO, J.S.H.¹; PEREIRA, G.²; BERGONZOLI, G.³; MATTA, L.⁴; RODRIGUEZ, J.⁵; DE ALMEIDA, D.C.². 1. UCEVA-COLOMBIA, TULUA - COLOMBIA; 2. UNIFESP, SAO PAULO - SP - BRASIL; 3. TOMAS URIBE HOSPITAL, TULUA - COLOMBIA; 4. UNIVALLE, CALI - COLOMBIA; 5. SOUTH COLOMBIAN UNIVERSITY, NEIVA - COLOMBIA.

Introduction: Dengue is a major arboviral disease caused by dengue virus (DENV), transmitted by *Aedes* mosquitoes. Between 2023 and 2024, the Americas experienced the largest recorded dengue epidemic. Exosomal microRNAs (miRNAs), transported in small extracellular vesicles, regulate immune responses in viral infections; however, their role in dengue severity remains unclear. **Objective:** To characterize exosomal miRNAs and immune markers associated with severe dengue in young hospitalized patients. **Methods:** A prospective study included young adults with laboratory-confirmed dengue, categorized as dengue without warning signs (DF, n=4) or dengue hemorrhagic fever (DHF, n=4). Blood samples were collected 24–48 h after admission. Cytokines were quantified by Cytometric Bead Array. Exosomes were phenotyped by multiparametric flow cytometry (CytoFLEX, Beckman Coulter), targeting CD14, CD86, and HLA-DR. RNA was extracted from serum exosomes, and miRNA profiling was performed via qPCR arrays. Bioinformatic tools identified altered pathways. Ethics approval No. 24-11-2020. **Results:** DHF patients showed elevated IL-12, TNF- α , IL-6, and increased leukocytes and monocytes. Flow cytometry revealed a greater proportion of CD14⁺ exosomes, including CD14⁺CD86⁺ and CD14⁺HLA-DR⁺ subtypes. Six miRNAs were upregulated in DHF, notably miR-15a-5p, which correlated positively with IL-6 and negatively with platelet count. Pathway enrichment indicated altered JAK-STAT, p53, and STAT1 signaling, suggesting dysregulation in type I interferon responses. **Conclusion:** miRNAs in exosomes, especially miR-15a-5p, combined with exosomal immune profiling, may serve as biomarkers for dengue severity. These findings support early clinical risk assessment and targeted immunomodulation. This is the first Colombian study integrating transcriptomic, exosomal, and immunological analysis in young dengue patients.

Keywords: Dengue;exosomes;microRNAs.

2911 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

AIR-LIQUID INTERFACE CULTURE MODULATES CYTOKINE PRODUCTION BY PULMONARY CELLS IN RESPONSE TO *Corynebacterium* spp.

BOTELHO, M.A.¹; ANDRADE-SILVA, M.²; SOUSA, A.M.¹; LIMA, M.V.D.S.¹; DURAO, C.V.¹; DE OLIVEIRA, E.E.¹; SILVA, F.M.D.C.E.¹; TORRES, E.J.L.¹; FRANÇA, F.L.D.S.¹; OLIVELLA, J.G.B.¹; SANT'ANNA, L.D.O.¹; SANT'ANNA, L.S.D.S.¹; DE MATTOS-GUARALDI, A.L.¹. 1. RIO DE JANEIRO STATE UNIVERSITY, RIO DE JANEIRO - RJ - BRASIL; 2. FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Background: The genus *Corynebacterium* comprises nearly 170 validly described species, many of which are human pathogens or pathobionts, such as *C. diphtheriae*, *C. ulcerans*, and *C. pseudodiphtheriticum*. Advances in cell culture techniques, particularly the air-liquid interface (ALI) method, have improved in vitro models by creating more physiologically relevant conditions. This study aimed to establish a protocol to investigate the immunological and microbiological effects of *Corynebacterium* spp. on lower respiratory tract cells. **Methods:** A549 cells (human alveolar epithelial cells, ATCC CCL-185) were cultured in Transwell inserts for 14 days under ALI or liquid-liquid interface (LLI) conditions. Cell morphology, ultrastructure and distribution were assessed by panoptic and fluorescent staining, histology, scanning (SEM) and transmission electron microscopy (TEM). The response to lipopolysaccharide (LPS) and interferon- γ (IFN- γ) was compared between models. Cells were challenged with *C. diphtheriae* ATCC 27010^T, *C. ulcerans* 809, and *C. pseudodiphtheriticum* 2802 (1×10^7 CFU/mL; 50 μ L). Interleukin (IL)-6, IL-10, IL-1 β and tumor necrosis factor α (TNF- α) were measured by enzyme-linked immunosorbent assay. *C. ulcerans* 809 was further tested for adherence and invasion in ALI, LLI, and conventional submerged cultures (2-3 days). **Results:** ALI cultures showed greater homogeneity, cell density, and a thicker layer compared to LLI. SEM and TEM revealed filopodia, closer cell-cell contact, and lamellar bodies in ALI. LPS/IFN γ induced a stronger inflammatory response in LLI, suggesting greater resistance in ALI. A similar effect was observed after challenge with *Corynebacterium* spp., with *C. ulcerans* inducing the highest IL-6 levels. However, no significant differences in adherence or invasion were observed between the culture models. **Conclusion:** ALI culture modulates the cytokine production by pulmonary epithelial cells but does not affect *C. ulcerans* 809 adherence or invasion.

Keywords: Air-liquid interface;Corynebacterium;Pulmonary epithelial cell.

2921 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Anti-Leishmania IgG Contributes to Inflammatory Response via FcγRs Engagement and predicts treatment failure in Cutaneous Leishmaniasis Patients

GUIMARÃES, J.M.C.S.; PEIXOTO, F.D.C.; COSTA, R.S.; DE CARVALHO, L.P.; CARVALHO, E.M.; CARVALHO, A.M.. FIOCRUZ-BAHIA, SALVADOR - BA - BRASIL.

Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is characterized by an exacerbated inflammatory response that contributes to parasite control but also leads to tissue damage and delays clinical cure. IgG antibodies can bind to Fc gamma receptors (FcγRs) on the surface of immune cells triggering the release of inflammatory cytokines. In this study, we prospectively evaluated whether anti-Leishmania antibodies can predict treatment failure with pentavalent antimony, the first-line therapy for CL in Brazil. In addition, we reanalyzed RNA-seq data from CL lesions focusing on plasma-cell differentiation (XBP1, MZB1, PRDM1), FcγRs signaling, and cytokine/cytotoxic mediators and evaluated the effect of FcγRs blockade on cytokine production in *L. braziliensis* lesions. Participants were CL patients (n = 21) with classical ulcers. Transcriptomic analyses revealed correlations between FcγRs expression and inflammatory genes (e.g., TNF, IL1B), cytotoxic genes (GZMB, PRF1), and IL10, suggesting a hybrid activation/regulatory phenotype. When lesion-derived tissue was cultured in the presence of an Fc receptor blocker, we observed a marked reduction in the production of IL-1β (80%), granzyme B (77%), and IL-10 (38%). Patients who failed therapy exhibited higher levels of anti-Leishmania IgG and the IgG1 subclass in the supernatants of *L. braziliensis* lesion cultures. Furthermore, a positive correlation was observed between anti-Leishmania IgG levels and healing time and receiver operating characteristic (ROC) analysis demonstrated that both anti-Leishmania IgG and IgG1 levels can predict treatment outcomes with high accuracy. Our findings indicate that anti-Leishmania antibodies contribute to inflammatory cytokine production and cytotoxic responses in CL through engagement of FcγRs and highlights their potential utility as predictive biomarkers of treatment response.

Keywords: Cutaneous leishmaniasis;Fc gamma receptors;B cells.

2928 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Design and Characterization of Dissolving Microneedles for Transdermal Delivery of a Neutralizing Monoclonal Antibody Against Zika Virus

ROJAS, J.E.U.¹; AZEVEDO, I.R.¹; DE SOUZA-SILVA, G.A.¹; ALMEIDA, B.D.S.²; YAMAMOTO, M.M.¹; SILVA, T.L.¹; DE OLIVEIRA, V.L.¹; BOSCARDIN, S.B.¹. 1. DEPARTAMENTO DE PARASITOLOGIA, INSTITUTO CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO, BRAZIL, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO/IMUNOTERA SOLUÇÕES TERAPÊUTICAS; 3. INSTITUTO DO CORAÇÃO (INCOR), SÃO PAULO - SP - BRASIL.

Introduction: Transdermal delivery of biologics represents a promising and less invasive alternative to conventional injections. This approach is particularly advantageous for needle-phobic patients and offers significant benefits in low-resource settings, where traditional methods might be impractical or challenging to administer.

Objective: This study aimed to design and characterize dissolving microneedles (MNs) for the transdermal delivery of a neutralizing monoclonal antibody (A9Z mAb) against the Zika virus.

Methodology: MN patches (10x10mm, 6x6 array, 1000µm needles) were fabricated via a two-step centrifugation casting method, using biocompatible polymers: hyaluronic acid (10%), PVP (50%), pullulan (20%), and Gantrez (20%). Characterization included: mechanical integrity, insertion studies (Parafilm®, porcine skin, OCT), dissolution tests (10-60 minutes), and trans-epidermal water loss (TEWL) for permeation potential. A9Z mAb incorporation and uniform distribution were confirmed by fluorescence microscopy (Alexa Fluor 488). Antibody structural stability post-formulation and storage was assessed by SDS-PAGE and qualitative fluorescence.

Results: MNs showed high structural integrity and successful skin penetration. Dissolution was rapid and complete within 10 to 60 minutes. TEWL confirmed temporary stratum corneum disruption, indicating permeation potential. The A9Z mAb was successfully incorporated and maintained its structural integrity after formulation and storage.

Conclusion: Dissolving polymeric MNs are a viable platform for transdermal delivery of high-molecular-weight therapeutics like neutralizing antibodies. This approach combines patient comfort, mechanical robustness, rapid dissolution, and formulation stability, representing a valuable strategy for non-invasive immunotherapies. This work is supported by FAPESP (#2022/14753-0 and # 2024/10604-5); CNPq (#307596/2023-0). Keywords: microneedles;skin delivery; monoclonal antibody.

2940 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Plasmodium yoelii infection modulates the expression of SIRP α -CD47 axis and associated cytokine response

DE OLIVEIRA, L.S.¹; GOMES, L.R.¹; MARTINS, P.D.C.¹; LIMA JUNIOR, J.D.C.²; DANIEL-RIBEIRO, C.T.¹; TOTINO, P.R.R.¹. 1. LABORATÓRIO DE PESQUISA EM MALÁRIA, IOC, FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. LABORATÓRIO DE IMUNOPARASITOLOGIA, IOC, FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

The SIRP α -CD47 axis is a relevant signaling pathway of the immune system and tissue homeostasis, participating in the regulation of phagocytosis, inflammatory immune response and lymphocyte activation. In experimental malaria, SIRP α -CD47 axis has been related to control of parasitemia as well as pathogenesis of the disease. However, knowledge about the role of the SIRP α -CD47 axis in malaria is still incipient. Thus, we studied the dynamics of SIRP α and CD47 splenic expression during the lethal (Py17XL) and non-lethal (Py17XNL) infection of *Plasmodium yoelii* in BALB/c mice, and the impact of SIRP α -CD47 axis blockage in the induced immune response. As expected, lethal infection was marked by hyperparasitemia, severe anemia, and intense hypothermia, all preceding mice death. Acute anemia was also observed in Py17XNL infection, which spontaneously resolved in parallel with parasitemia. Both strains negatively modulated SIRP α expression on splenocytes, with slight effect on CD47, and a significant increase in the frequency of SIRP α + cells was noted only during non-lethal infection. Interestingly, *in vitro* antibody blockade of the SIRP α -CD47 pathway reduced cytokine secretion by splenocytes obtained from non-lethal infection, while an increase was observed in lethal infection, especially of IL-6. In conclusion, our results suggest that the expansion of SIRP α + cells may be involved in the resistance to lethal malaria infection and that *Plasmodium* strains can differently impact the cytokine response mediated by SIRP α -CD47 signaling, as observed under antibody blocking treatment. However, further studies are still needed to better understand the role of SIRP α -CD47 axis in the immunopathogenesis of malaria. Keywords: Malaria;P; yoelii;SIRP α -CD47 .

2948 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Characterization of the feline macrophage cell line Fcwf-4 treated with glucuronoxylomannan (GXM) and infected by the parasite Leishmania amazonensis.

MOREIRA, F.D.P.P.¹; BELARMINO, N.D.L.¹; DA SILVA, L.P.¹; DIAS, T.D.S.¹; DOS SANTOS, R.Q.¹; ABRANTES, L.S.¹; PREVIATO, L.M.²; PREVIATO, J.O.²; DA SILVA JUNIOR, E.B.²; FREIRE-DE-LIMA, C.G.²; DECOTE-RICARDO, D.¹; DA SILVA, L.H.P.¹. 1. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, SEROPEDICA - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis is a disease caused by parasites of the genus *Leishmania*, transmitted to humans and animals through the bite of infected sandflies. Although it predominantly affects humans and dogs, studies suggest that immunosuppression may make felines more susceptible to *Leishmania* infection and they may be secondary reservoirs of the disease. This study aimed to evaluate the interaction between the parasite *Leishmania amazonensis* (*L. a.*) and a feline macrophage cell line (Fcwf-4) *in vitro*. We analyzed if the presence of the immunomodulatory molecule, the fungal polysaccharide glucuronoxylomannan (GXM) from *Cryptococcus neoformans*, could immunomodulate Fcwf-4 during *Leishmania* infection. First, GXM [100 μ g/mL] toxicity analysis showed that cell viability was not altered in *L. a.*-feline macrophages infected or not. Feline macrophages were infected with *L. a.* in the presence or not of GXM. The data demonstrated that Fcwf-4 can phagocytose *L. a.*-promastigotes and after 2 hours of interaction, it was found 62% of *L. a.*-infected feline macrophages, however, in the presence of GXM, it was observed 49.03% of it. On the other hand, at 48 hours in the presence of GXM 24% of *L. a.*-infected macrophages were found, while in control remained in 10 % of *L. a.*-infected macrophages. Regarding the parasite load, preliminary results showed that GXM-treated feline macrophages have 41x10⁶/ml parasites, while untreated cells have 20x10⁶/ml. Nitric oxide (NO) production showed no difference in GXM-untreated and treated cells, or stimulated with 400ng/ml of LPS compared to control. Preliminary data showed an increase around 1,2 times in ROS production in feline macrophages stimulated with LPS or GXM or *L. a.*-infected cells in the presence of GXM and LPS compared to control. Next, it will evaluate cytokine profiles and the expression of MHC II molecules in these cells. To comprehend the feline macrophage biology is essential for understanding immune responses and disease mechanisms.

Keywords: feline macrophage;leishmaniasis;glucuronoxylomannan (GXM).

2950 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Dynamics of B cell subpopulations and immunoglobulin repertoire during experimental infection with *Plasmodium yoelii* 17XNL

ANTONIO, N.D.S.; DE SOUZA-SILVA, G.A.; GAITÁN, X.A.; AZEVEDO, I.R.; YAMAMOTO, M.M.; DE OLIVEIRA, T.R.; SOARES, I.D.S.; BOSCARDIN, S.B.; SULCZEWSKI, F.B.. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Malaria, caused by *Plasmodium* parasites, is a major global health burden. While B lymphocytes and antibodies are crucial for controlling parasitemia, infection alters B cell function, potentially leading to dysregulated immune responses and autoreactive antibodies. Understanding these dynamics is essential for elucidating protective immunity and pathogenic mechanisms. This study aimed to characterize the dynamic changes in B lymphocyte subpopulations and the antigen-specific antibody repertoire following *Plasmodium yoelii* 17XNL infection in a non-lethal murine model. To evaluate antigen-specific responses, the MSP1₁₉ protein was recombinantly expressed in bacteria and validated by Western blot. In initial experiments, C57BL/6 mice were intraperitoneally infected with 1×10^6 *P. yoelii* 17XNL-infected red blood cells (iRBCs) or healthy RBCs as controls. Parasitemia was monitored daily via green fluorescent protein (GFP) expression by flow cytometry. For comprehensive B cell subset analysis, splenocytes were harvested at 10 days post-infection (p.i.), stained with specific surface and intracellular markers, and analyzed using flow cytometry. Parasitemia peaked approximately 5 days p.i. and gradually declined, leading to complete infection control by day 20. Infected mice exhibited significant splenomegaly. Flow cytometric analysis revealed an increase in the frequency and absolute number of various B lymphocyte subsets in infected mice compared to uninfected controls. Notably, we identified an expanded population of atypical memory B cells (AtyMBC) characterized by the co-expression of CD11c and T-bet, consistent with observations in human malaria. Furthermore, a significant fraction of these AtyMBC also co-expressed FAS and GL7. These findings demonstrate significant expansion and phenotypic alteration of B lymphocyte populations, including atypical memory B cells, during experimental *P. yoelii* 17XNL infection.

Keywords: Antibody; Plasmodium; B lymphocyte.

2952 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Identification of miRNA Signatures Associated with Resistance to SARS-CoV-2 in Clinically Discordant Couples

DA SILVA, L.I.¹; FERREIRA, T.R.¹; CARVALHO, M.¹; CASTRO, C.F.²; DIAS-MELICIO, L.A.¹. 1. SÃO PAULO STATE UNIVERSITY - UNESP, BOTUCATU - SP - BRASIL; 2. SÃO PAULO STATE UNIVERSITY - UNESP, BRAZIL - SP - BRASIL.

Discordant couples, in which one partner becomes infected with SARS-CoV-2 while the other remains uninfected despite close and prolonged exposure, represent a valuable natural model for studying resistance to COVID-19. Growing evidence indicates that host genetic and immune-related factors, including post-transcriptional regulators such as microRNAs (miRNAs), may contribute to this resistance. This study aimed to investigate the differential expression of miRNAs in individuals exposed to SARS-CoV-2 who remained uninfected compared to their infected partners, in order to identify miRNA signatures potentially associated with natural resistance to infection. Plasma samples were collected from 8 clinically characterized discordant couples. MicroRNAs were extracted and analyzed using the NanoString nCounter® Human v3 miRNA Expression Assay, which enables direct and multiplexed quantification of over 800 human miRNAs without amplification. A total of 18 differentially expressed miRNAs were identified, with two upregulated ($p < 0.05$; $FC > 1.5$) and 16 downregulated ($p < 0.05$; $FC < -1.5$) in exposed but uninfected individuals compared to their infected partners. These distinct miRNA expression patterns in naturally resistant individuals, along with enriched pathways related to cell cycle regulation, signal transduction, and immune modulation, suggest a protective role for miRNA-mediated regulatory mechanisms in resistance to SARS-CoV-2 infection. Our findings highlight the potential of miRNAs not only as biomarkers of resistance but also as promising molecular targets for therapeutic strategies. Financial Support: FAPESP process n. 2021/13354-1.

Keywords: SARS-CoV-2 ;microRNAs (miRNAs);resistance against infection.

2957 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Echinococcus granulosus antigen B: a parasite lipoprotein with immunoregulatory and lipid acquisition properties in myeloid cells

FOLLE, M.¹; MAGALLANES, S.L.¹; BEASLEY, A.¹; ZAMARREÑO, F.²; CARRIÓN, F.³; FLÓ, M.³; COSTABEL, M.⁴; MACCIONI, M.⁵; JULVE, J.⁶; FERREIRA, A.M.¹. 1. UNIVERSIDAD DE LA REPÚBLICA, MONTEVIDEO - URUGUAI; 2. UNIVERSIDAD NACIONAL DE SUR, BAHÍA BLANCA - ARGENTINA; 3. INSTITUTO PASTEUR DE MONTEVIDEO, MONTEVIDEO - URUGUAI; 4. UNIVERSIDAD NACIONAL DEL SUR, BAHÍA BLANCA - ARGENTINA; 5. UNIVERSIDAD NACIONAL DE CÓRDOBA, CÓRDOBA - ARGENTINA; 6. INSTITUT DE RECERCA SANT PAU, BARCELONA - ESPANHA.

The larval stage of *Echinococcus granulosus*, responsible for cystic echinococcosis, establishes a chronic infection that tightly modulates host immunity. Adapted to a nutrient-rich environment, the parasite has lost its de novo synthesis of fatty acids and cholesterol, relying on host-derived lipids via specialized proteins. Antigen B (EgAgB), the main larval lipoprotein belonging to the cestode-specific hydrophobic ligand-binding protein family, is exported to host tissues and resembles HDL in physicochemical properties and anti-inflammatory actions on innate cells.

Our investigation on EgAgB's biological activities revealed that both native and recombinant lipoproteins induced a modest dendritic cell (DC) activation, attributed to LPS contamination, while suppressing LPS-induced cytokine (IL1 β , IL6, IL12, IFN β) and nitric oxide production in DC and macrophages (MQ) by disrupting TLR4 dimerization. In the current work, we confirmed that in an LPS stimulation context EgAgB does not affect ATP potentiation of IL1 β secretion linked to inflammasome activation in MQ. Moreover, EgAgB outcompeted LPS for binding to DC/MQ and inhibited LPS-driven cytokine release more effectively than HDL. Binding assays and light scattering approaches confirmed EgAgB's superior LPS-binding ability, supported by docking analysis showing a defined LPS-binding interface in EgAgB8/1 subunit.

Regarding lipid handling, EgAgB acquired cholesterol from HDL and MQ, suggesting additional interactions with host cells that may have functional relevance and are currently under investigation.

Our findings reveal novel activities for EgAgB: an extracellular LPS scavenger property that dampens TLR4-mediated inflammation in myeloid cells and a cholesterol uptake capacity from host lipoproteins/cells. Altogether, results support a dual role of EgAgB in *E. granulosus* biology, contacting host components to acquire essential lipids while contributing to parasite protection from host inflammation.

Keywords: Echinococcus granulosus antigen B; Immunomodulation; Lipid metabolism.

2965 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Omega-3 modulates Macrophage cytokine production and glucose metabolism toward a hyporesponsive state without changing Pseudomonas aeruginosa proliferation

AUGUSTO, L.V.; SILVA, E.N.; DE ARAÚJO, L.P.; BARROS, N.; DE OLIVEIRA, L.M.G.; DE MIRANDA, T.T.; DE ALMEIDA, L.A.; CORSETTI, P.P.. UNIFAL - MG, ALFENAS - MG - BRASIL.

The increasing and often indiscriminate use of medications challenges populations with limited access to effective treatments. Natural compounds like omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have immunomodulatory effects by incorporating into macrophage membranes, modulating cytokine production and metabolism. This study evaluated omega-3 effects on cytokine production and glycolytic metabolism in bone marrow-derived macrophages (BMDMs) infected with *Pseudomonas aeruginosa*. BMDMs from C57BL/6 mice were pretreated for 3 hours with 26 mg DHA and 36 mg EPA, then stimulated with *P. aeruginosa* (PA14), heat-killed bacteria (HKPA), or lipopolysaccharide (LPS). Omega-3 pretreatment reduced pro-inflammatory cytokines TNF- α , IL-1 β , and nitric oxide in macrophages stimulated with PA14 and HKPA, while IL-10 secretion was also reduced under these stimuli. However, macrophages pretreated with omega-3 showed increased IL-10 production depending on the stimulus. Treated macrophages showed increased glucose consumption and lactate release, indicating metabolic reprogramming toward a hyporesponsive state. Omega-3 did not affect *P. aeruginosa* proliferation, suggesting microbial control was maintained. Molecular docking suggests omega-3 fatty acids interact with key inflammatory targets, such as GPR120 and PPAR γ . These results indicate omega-3 modulates macrophage immune response and metabolism during *P. aeruginosa* infection, promoting an anti-inflammatory, metabolically adapted phenotype without impairing bacterial clearance.

Keywords: Fatty acids; Immunometabolic regulation; PA14.

2978 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Fc-Mediated Antibody Functions Against SARS-CoV-2: The Role of ADCP and Non-Spike Antigens in Protective Immunity

SILVA, N.B.¹; BENTLEY, K.²; SAUD, Z.²; FIELDING, C.A.²; DOORES, K.³; PRESTON, H.²; FARIAS, A.D.S.⁴; STANTON, R.J.². 1. UNIVERSITY OF CAMPINAS - UNICAMP, CAMPINAS - SP - BRASIL; 2. CARDIFF UNIVERSITY, CARDIFF - PAIS DE GALES; 3. KING'S COLLEGE LONDON, LONDON - INGLATERRA; 4. UNIVERSITY OF CAMPINAS-UNICAMP, CAMPINAS - SP - BRASIL.

The emergence of several variants of SARS-CoV-2 with mutations mainly in the Spike protein and the short duration of the neutralizing activity of the antibodies generated by the different vaccine formulations are a prominent reason for global concern. Nevertheless, the decrease in neutralizing protection is incompatible with the frequency of reinfections or severe cases in vaccinated individuals. Beyond neutralization, antibodies also exert Fc-mediated functions that aid in host protection. Among them, antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) contribute to viral clearance through killing and removal of infected cells and are still underexplored in SARS-CoV-2 context. Here, we show that the SARS-CoV-2-infected cells are susceptible to ADCP and evaluate this mechanism in the face of vaccine platforms, disease severity, and hybrid immunity. Sera from individuals with severe COVID-19 exhibited higher ADCP responses than those with mild disease, while those from vaccinated individuals, regardless of vaccine type, were comparable to patients with severe disease. Notably, hybrid immunity induced stronger and more sustained phagocytic responses across dilutions. Within mRNA-vaccinated individuals, ADCP was entirely directed against the Spike protein. However, following natural infection, non-Spike antigens, such as ORF3a and membrane proteins, were equally important in driving ADCP activity. To assess whether the viral evolution can impact this Fc-mediated function, we evaluated ADCP in cells infected with the Omicron BA.1 variant. Furthermore, ADCP and ADCC are driven differently across the cohorts analysed. In this way, our findings highlight the relevance of non-Spike antigens in contributing to phagocytic responses and the importance of their inclusion in next-generation vaccine designs to broaden antibody-mediated protection and avoid immune escape due to SARS-CoV-2 evolution.

Keywords: SARS-CoV-2;Antibody-Dependent Cellular Phagocytosis;Humoral Immunity.

2980 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Exhaustion on *Leishmania amazonensis* and *Leishmania braziliensis* infection

PORCINO, G.N.¹; COVRE, L.P.²; GUEDES, H.L.D.M.¹; MARTINS, A.M.D.F.¹. 1. INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES - UFRJ / INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. NÚCLEO DE DOENÇAS INFECIOSAS, UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL.

Chronic antigenic stimulation can lead to the induction of T cell exhaustion, which hampers the T cell response necessary for controlling the infectious process. In the case of infection with *Leishmania amazonensis* in mice, a suppressive capacity is observed, wherein the expression of PD-1 on lymphocytes and PD-L1 on macrophages, neutrophils, and dendritic cells contributes to the failure in generating an effective immune response. The infection by *L. amazonensis* triggers the expression of PD-L1 on these cells, indicating that the parasite exploits this pathway to evade the immune response. Treatment with anti-PD1 or anti-PD-L1 antibodies can control the parasite load by reinvigorating the CD4⁺ and CD8⁺ T cell responses and promoting increased production of interferon-gamma. On the other hand, infection with *L. braziliensis* exhibits the ability to induce an effective immune response capable of controlling the parasite load. However, when analyzing the infection by *L. braziliensis*, it is also observed that the parasite induces PD-L1 expression both *in vitro* and *in vivo*. Treatment with anti-PD1 and anti-PD-L1 antibodies *in vivo* enhances the control of the parasite load, indicating that the exhaustion process also occurs in *Leishmania braziliensis* infection. Furthermore, immunotherapy has the potential to improve the ability to control the parasite load by enhancing the interferon-gamma response. These findings support previous data obtained from patients with cutaneous leishmaniasis caused by *Leishmania braziliensis* and suggest the possibility of using immunotherapy in patients who do not respond well to conventional treatments.

Keywords: *Leishmania amazonensis*; *Leishmania braziliensis*; exhaustion.

2989 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

ROLE OF ATPASE INHIBITORY FACTOR 1 (IF1) IN CD4⁺ T CELLS DURING THE ACUTE PHASE OF PLASMODIUM INFECTION

DE SOUZA, D.C.D.S.R.¹; CODOGNATO, S.G.¹; DO NASCIMENTO, S.R.¹; FREITAS, R.¹; MANHÃES, L.D.S.B.¹; MATTOS, G.H.R.¹; CIONE, M.V.P.¹; DO NASCIMENTO, R.S.¹; RAEDER, P.H.L.¹; CANTARINI, D.G.¹; RUIZ, D.F.²; HEATH, W.²; ALVAREZ, J.M.¹; LIMA, M.R.D.¹. 1. INSTITUTE OF BIOMEDICAL SCIENCES - UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. THE PETER DOHERTY INSTITUTE FOR INFECTION AND IMMUNITY - THE UNIVERSITY OF MELBOURNE, MELBOURNE - AUSTRALIA.

ATPase Inhibitory Factor 1 (IF1) is a protein that acts as the endogenous inhibitor of mitochondrial ATP synthase. In various cell types, there is increasing evidence that IF1 activity influences cell metabolism, activation and survival; however, the role of IF1 in CD4⁺ T cells is still poorly understood. To fill this gap, TCR-specific CD4⁺ T cells were transferred to C57BL/6 wild-type (B6) mice infected with *P. chabaudi*. Analysis of public single-cell RNA-seq data revealed increased IF1 gene expression following infection; these results were confirmed by RT-qPCR up to day 7 post-infection (p.i.). On day 5 p.i., disrupting IF1 gene expression on these cells did not affect the phosphorylation of metabolic sensors Akt and mTOR, responsiveness to metabolic inhibitors 2-DG and oligomycin, mitochondrial polarization and expression of proliferative marker Ki-67; however, the population of T_H1 cells was increased, suggesting that IF1 expression may impair T_H1 differentiation. Similar results were found when IF1-deficient cells were transferred to C57BL/6 CD4 knockout (*Cd4^{-/-}*) mice. To evaluate whether IF1 expression in CD4⁺ T cells contributes to the development of severe manifestations of malaria, IF1-deficient cells were transferred to *P. berghei* ANKA-infected *Cd4^{-/-}* mice. Splenic IF1-deficient cells showed increased numbers and T-bet expression, but no differences were observed concerning this population in the brain. Surprisingly, mice transferred with IF1-deficient CD4⁺ T cells showed increased expression of TMEM-119 in microglial cells, suggesting less reactive microglia upon IF1 deficiency in CD4⁺ T cells. Taken together, these results may indicate that IF1 controls CD4⁺ T cell response differently in the spleen and brain of mice infected with *Plasmodium* parasites. Further experiments are needed to confirm these findings. This project is supported by FAPESP (2022/14529-2; 2015/20432-8), CAPES (88887.820673/2023-00) and CNPq (303810/2018-1).

Keywords: IF1;CD4⁺ T cells;malaria.

2997 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Axl deletion enhances the expression of co-stimulatory molecules in dendritic cells in response to BCG infection

LOIOLA, L.H.M.; DE ANDRADE, A.C.M.M.; COSTA, D.L.. INSTITUTO DE CIÊNCIAS BIOMÉDICAS DA UNIVERSIDADE DE SÃO PAULO - DEPARTAMENTO DE IMUNOLOGIA, SÃO PAULO - SP - BRASIL.

BCG is the only vaccine approved for human use to prevent tuberculosis (TB). Although effective in protecting newborns against disseminated disease, the vaccine fails to protect adults against the pulmonary form of TB. In this sense, a vaccine that can induce long-lived lung-resident memory CD4⁺ T cells may be able to provide better protection against TB in adults. The activation of Axl and MerTK receptors, which are widely expressed in mononuclear phagocytes, negatively regulates TLR signaling as well as pro-inflammatory cytokine production and co-stimulatory molecule expression, indirectly suppressing T lymphocyte activation. We hypothesize that blocking Axl and MerTK receptors in dendritic cells (DCs) might enhance BCG efficacy in promoting memory CD4⁺ T cell responses, thereby enhancing protection against *Mycobacterium tuberculosis* (Mtb) infection. In order to start evaluating this possibility, we have first characterized whether the deletion of Axl or MerTK interferes with cytokine production and co-stimulatory molecules expression in bone marrow-derived DCs 24h post BCG infection in vitro. Using flow cytometry, we found that CD40 expression was similar across groups, but CD86⁺ and MHCII⁺ cells were higher in Axl^{-/-} compared to C57BL/6 and MerTK^{-/-} groups. We have also quantified IL-1β, TNF and IL-12 in culture supernatants by ELISA, but found no differences between C57BL/6, Axl^{-/-} and MerTK^{-/-} DCs. These results suggest that blocking Axl may be effective in improving the efficacy of DCs to present BCG antigens to T CD4⁺ T cells, without affecting pro-inflammatory cytokine production. In vivo BCG vaccination experiments using C57BL/6, Axl^{-/-}, and MerTK^{-/-} mice with subsequent Mtb challenge are ongoing, which will help us to determine if indeed the absence of Axl signaling might enhance antigen-presenting capacity of DCs, impacting their potential to induce CD4⁺T cell memory responses and protection against experimental TB. Financial Support: FAPESP and CNPq.

Keywords: BCG;AXL / MERTK;M; tuberculosis.

3000 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Higher T cell activation and exhaustion during SARS-CoV-2 acute infection in people living with HIV-1 admitted at a hospital in Rio de Janeiro, Brazil

SOUZA, L.R.¹; DE CASTRO, C.¹; ABRAHÃO, M.M.¹; DE SÁ, N.B.R.¹; GOULART, M.N.G.¹; CAZOTE, A.D.S.¹; DE ALMEIDA, D.V.¹; GRIPP, C.B.W.G.¹; ROSADAS, L.A.D.S.²; GERALDO, K.M.²; RIBEIRO, M.P.D.²; CARDOSO, S.W.²; GRINSZTEJN, B.²; VELOSO, V.G.²; MORGADO, M.G.¹; CÔRTEZ, F.H.¹. 1. FIOCRUZ/IOC, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ/INI, RIO DE JANEIRO - RJ - BRASIL.

Although COVID-19 severity in people living with HIV (PLWH) remains debated, studies suggest that HIV infection leads to a worse progression of COVID-19, mainly in those with low CD4 counts. Since higher T cell activation and exhaustion are observed in both HIV infection and severe Covid-19, the aim of this study was to investigate whether this profile was exacerbated during SARS-CoV-2/HIV-1 co-infection.

PBMC from 11 people with SARS-CoV-2 and 11 PLWH coinfecting with SARS-CoV-2, all hospitalized, were stimulated with SARS-CoV-2 protein S peptides. To identify T cell activation, exhaustion, and SARS-CoV-2-specific T cells, the samples were stained with anti-CD3, CD4, CD8, PD-1, HLA-DR, CD134, CD137, CD69 antibodies. Then, samples were acquired in a flow cytometer and analyzed in FlowJo.

We found a higher frequency of CD4⁺ T cells expressing activation (med=4.76 vs. 0.73, $p<0.0001$) and exhaustion (med=28.90 vs. 4.9; $p<0.0001$) markers in the coinfecting group compared to the monoinfected group. The same profile was observed in CD8⁺ T cells, with higher activation (med=10.10 vs. 1.51; $p=0.015$) and exhaustion (med=14.40 vs. 3.98; $p=0.01$) markers in the coinfecting group. Although there was no statistical difference in the percentage of SARS-CoV-2-specific T cells, we observed a higher frequency of responders among those who were discharged and in the coinfecting group, mainly in CD8⁺ T cells, which were associated with lower activation and increased survival. We also observed higher frequency of CD8⁺ T cells in discharged people compared to those who died (med=23.15 vs. 11.90; p value=0.01). We are currently recruiting PLWH without SARS-CoV-2 infection to form our second control group.

The HIV infection was linked to higher T cell activation and exhaustion during COVID-19. The higher frequency of total CD8⁺ T and SARS-CoV-2-specific T cells was associated with survival, suggesting a potential protective role of these cells in COVID-19.

Keywords: T cells; SARS-CoV-2; HIV-1.

3001 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

OMEGA-3 DHA MITIGATES NEUROINFLAMMATION AND METABOLIC DYSREGULATION DURING MICROGLIA ZIKV INFECTION

LAGO, F.G.¹; DE MELO, H.A.B.¹; CORREA, R.²; BELLOZI, P.M.Q.³; ALMEIDA, R.D.N.³; DE BEM, A.F.³; BAO, S.N.³; RIBEIRO, B.M.³; WILLIAMS, T.C.³; MAGALHÃES, K.G.⁴. 1. LABORATORY OF IMMUNOLOGY AND INFLAMMATION – UNIVERSITY OF BRASÍLIA (UNB), BRASÍLIA - DF - BRASIL; 2. LABORATORY OF MOLECULAR IMMUNOLOGY - UNB, BRASÍLIA - DF - BRASIL; 3. UNIVERSITY OF BRASÍLIA, BRASÍLIA - DF - BRASIL; 4. LABORATORY OF MOLECULAR IMMUNOLOGY- UNB, BRASÍLIA - DF - BRASIL.

Zika virus (ZIKV) is a neurotropic arbovirus associated with microglia-mediated neuroinflammation and metabolic dysregulation in the central nervous system. Previous works from our group have demonstrated the neuroprotective and antiviral effects of omega-3 docosahexaenoic acid (DHA) in neuronal-like cells. Therefore, this project aimed to investigate the role of DHA through metabolic disturbances that define the ZIKV infection in microglia.

METHODS: Human microglial cells were pre-treated with DHA (12.5 μ M) and infected with ZIKV. Cell viability was evaluated using MTT and LDH assays. Cytokine levels were quantified by ELISA, and RT-qPCR determined viral load. Cell proliferation and activation parameters were assessed through CFSE and BODIPY staining. The expression of AXL was analyzed by flow cytometry. Mitochondrial dynamics were evaluated using high-resolution respirometry, confocal microscopy, and scanning electron microscopy. Microglial morphology and lipid droplet (LD) accumulation were analyzed by confocal microscopy. Finally, the metabolic profile was determined using gas chromatography-mass spectrometry.

RESULTS: Our data showed that DHA can mitigate ZIKV-induced microglial activation by reducing LD accumulation and cytoskeletal alterations. Moreover, DHA attenuates the pro-inflammatory state and promotes an anti-inflammatory response. When microglial metabolism was evaluated, DHA protected microglia against ZIKV-related changes by maintaining levels of malate and fumarate and inducing higher levels of intracellular lactate. Beyond that, DHA mitigated the increase of smaller mitochondria in the perinuclear region and preserved both spare and maximal respiratory capacity. Finally, DHA lowered AXL expression and ZIKV RNA levels.

CONCLUSION: These findings suggest that DHA's antiviral and anti-inflammatory effects may be linked to its ability to modulate neuroinflammation and pathogenesis during ZIKV infection, underscoring its potential as a therapeutic strategy.

Keywords: DHA; ZIKV; MICROGLIA.

3008 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

CD39 Downregulates Pulmonary Inflammation in Malaria-Associated Acute Respiratory Distress Syndrome

MANHÃES, L.D.S.B.¹; CIONE, M.V.P.¹; FREITAS, R.¹; DE SOUZA, D.C.D.S.R.¹; DA FONSECA, D.L.M.¹; BAHIA, I.A.F.¹; CODOGNATO, S.G.¹; MATTOS, G.H.R.¹; CANTARINI, D.G.¹; DO NASCIMENTO, R.S.¹; CLASER, C.²; MARINHO, C.R.F.¹; COUTINHO-SILVA, R.³; ROBSON, S.C.⁴; 22/00291-4, S.M.M.F.¹; ALVAREZ, J.M.¹; LIMA, M.R.D.¹. 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 4. HARVARD MEDICAL SCHOOL, BOSTON - ESTADOS UNIDOS DA AMERICA.

Malaria-associated acute respiratory distress syndrome (ARDS-MA) causes severe pulmonary damage and elevates extracellular ATP (eATP) levels. eATP can activate purinergic P2 receptors, amplifying inflammation, or be hydrolyzed by the ectonucleotidases CD39 and CD73 into adenosine, which promotes anti-inflammatory signaling. Here, we investigated the role of CD39 in ARDS-MA using a murine model of *Plasmodium berghei* NK65 infection (10⁶ iRBCs). On days 6 and 7 post-infection (p.i.), wild-type (WT) and CD39-deficient (*Entpd1*^{-/-}) mice exhibited comparable mortality, weight loss, clinical scores, and parasitemia (18–25%). However, by day 6 p.i., *Entpd1*^{-/-} mice presented increased lung weight and enhanced cellular infiltration, particularly of T cells. Histopathological analysis revealed exacerbated pulmonary edema and a higher number of activated CD4⁺ T cells (CD4⁺CD11a⁺), with a modest increase in IFN- γ production. No significant differences were observed in the CD11a⁺CD8⁺ T cell population. In WT mice, CD39 expression was upregulated in activated CD4⁺ and CD8⁺ T cells in the lungs at day 6 p.i. To further explore these findings, we analyzed a public single-cell RNA-seq lung data (GSE244528) from WT mice with ARDS-MA using Seurat in R. UMAP clustering (resolution 0.1) identified 11 distinct cell populations, including CD3⁺ T cells, which exhibited upregulation of *Entpd1* during infection. Increased *Entpd1* expression was also observed in endothelial cells, while a downregulation was detected in neutrophils. Within the CD3⁺ T cell cluster, *Entpd1* expression was enriched in cytotoxic, effector, and regulatory T cell subsets. These findings suggest that CD39 downregulates CD4⁺ T cell activation during ARDS-MA and may also contribute to endothelial and neutrophil modulation in the pulmonary immune response. This research is supported by FAPESP (2023/10839-0; 2022/00858-4; 2015/20432-8), CAPES (88887.659095/2021-00), and CNPq (308875/2023-0).

Keywords: Pulmonary malaria; Ectonucleotidase CD39; *Plasmodium berghei* NK65.

3010 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Comparative analysis of distinct sheep-associated staphylococci and mammaliococci species in resisting phagocytosis and triggering intracellular ROS production by blood PMNLs

RUAS, Y.F.¹; MARQUES, M.F.S.²; DE MOURA, G.S.²; FERRONATTO, J.A.¹; LIBERA, A.M.M.P.D.¹; GARGANO, R.G.¹; HEINEMANN, M.B.¹; MOTA, R.A.²; DE SOUZA, F.N.¹. 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO, RECIFE - PE - BRASIL.

Mastitis is a major cause of economic losses and reduced animal welfare in dairy sheep, often involving staphylococci and mammaliococci species. This study aimed to compare the ability of different staphylococci and mammaliococci species, isolated from distinct ecological niches of sheep and lambs, to evade phagocytosis and modulate reactive oxygen species (ROS) production by blood polymorphonuclear leukocytes (PMNLs). These bacteria are known to colonize or infect ruminants and may vary in their capacity to interact with the host immune system depending on their ecological origin. Twenty-five isolates from clinical and subclinical mastitis, ewe teat skin, and lamb tonsils were analyzed using flow cytometry. CFSE-labeled viable bacteria were incubated with blood PMNLs to assess phagocytosis, while ROS production was evaluated using DCFH-DA probe. Both opsonized and non-opsonized bacteria were assessed to simulate different immune conditions. Opsonization significantly increased the phagocytosis percentage for most species, particularly *Mammaliococcus sciuri* and *Staphylococcus haemolyticus*, although GMFI values were often higher in the non-opsonized group. *S. devriesei* showed the lowest phagocytosis rates, while *S. xylosus* triggered the highest GMFI and ROS production among all species. *S. aureus* and *S. haemolyticus* exhibited a greater ability to evade phagocytosis. The ecological niche influenced phagocytic response: isolates from clinical mastitis were more phagocytosed than those from subclinical cases, suggesting a differential recognition or immune activation. However, no bacterial species inhibited ROS production; all induced levels were equal to or higher than those of the unstimulated control. These findings suggest that bacterial species and their ecological origin distinctly modulate innate immune activation, with potential implications for pathogenesis and persistence of ovine intramammary infections.

Keywords: Mastitis; *Staphylococcus aureus*; innate immunity.

3034 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Deletion of AhR in Myeloid Cells Impairs *Leishmania amazonensis* Control and Promotes Tissue Damage

MARTINS, A.J.E.¹; SANTOS, W.G.S.¹; DOS SANTOS, T.P.¹; DA SILVA, G.R.¹; BARBOSA, M.¹; CASTELUCCI, B.G.¹; DOS REIS, L.M.¹; BRIOSCHI, M.B.C.²; DE OLIVEIRA FILHO, V.A.²; MIGUEL, D.C.²; VIEIRA, P.M.M.D.M.¹. 1. LABORATORY OF IMMUNOMETABOLISM, UNICAMP, CAMPINAS - SP - BRASIL; 2. LABORATORY OF LEISHMANIA INFECTION BIOLOGY STUDIES, UNICAMP, CAMPINAS - SP - BRASIL.

Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*, with *L. amazonensis* being prevalent in Brazil. These parasites adapt to the intracellular environment, making the study of immunometabolic aspects essential. The aryl hydrocarbon receptor (AhR) regulates macrophage responses by influencing arginine metabolism and immune responses to trypanosomatids; however, its role in infections caused by *L. amazonensis* remains unexplored. To investigate the function of AhR in this context, bone marrow-derived macrophages from AhRFloxLysMcre (AhRKO) and AhRFlox (control) mice were cultured and infected with *L. amazonensis*. In vitro experiments demonstrated that infection results in reduced levels of AhR protein and decreased expression of its target gene Ahrr. Deletion of AhR resulted in increased Arg1 and decreased NOS2 and NOX2 expression, which was accompanied by higher parasite burden at 24 hours post-infection. These findings suggest that AhR plays a key role in the initial macrophage response to the parasite. Next, AhRKO and AhRFlox mice were infected with *L. amazonensis* amastigotes for either 5 or 12 weeks, and the progression of infection was monitored. Accelerated lesion development and higher parasite loads were observed in AhRKO mice during the early phase of infection. After 12 weeks, although parasite burdens were comparable between groups, AhRKO animals exhibited impaired tissue organization and altered collagen distribution. These results indicate that the absence of AhR in the early stages of infection compromises the microbicidal response and contributes to tissue damage, reinforcing the importance of AhR in host defense against *L. amazonensis*.

Keywords: immunometabolism; immune response; Macrophages.

3047 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Comparative Immunoenzymatic Analysis of IgG and IgM Peptides from the *Leptospira* LipL49 Protein for the Development of Novel Immunological Diagnostic Tests for Leptospirosis

TAPAJÓZ, R.C.D.S.¹; DELGADO, M.V.B.¹; BALASSIANO, I.T.²; DA SILVA, F.R.¹; DE SIMONE, S.G.¹. 1. NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY FOR INNOVATION IN DISEASES OF NEGLECTED POPULATIONS (INCT-IDPN)/CENTER FOR TECHNOLOGICAL DEVELOPMENT IN HEALTH (CDTS) - OSWALDO CRUZ FOUNDATION (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 2. NATIONAL REFERENCE LABORATORY FOR LEPTOSPIROSIS (LRNL)/OSWALDO CRUZ INSTITUTE (IOC) - OSWALDO CRUZ FOUNDATION (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

The objective of this study was to comparatively analyze the IgG and IgM responses through two ELISA tests, NeutrAvidin™ ELISA and indirect ELISA, using biotinylated synthetic peptides of the *Leptospira* LipL49 protein as antigens. ELISA sensitization: 1 µg peptide; 1st antibody: 1:100-IgG/1:50-IgM; 2nd antibody: 1:30,000-IgG/1:15,000-IgM; Absorbance measurement: 450 nm. For IgG peptides, a statistically significant difference was observed in the reactivity of the LipL49/6G peptide in the NeutrAvidin ELISA between all the groups with negative controls: <6 days and non-reactive control (*), <6 days and syphilis control (**), 7-13 days and non-reactive control (**), 7-13 days and syphilis control (**), >14 days and non-reactive control (***), >14 days and syphilis control (*). In the indirect ELISA, the same peptide showed the same significance in the groups <6 days and syphilis control, 7-13 days and non-reactive control, and >14 days and syphilis control (**). For the LipL49/12G peptide, in the NeutrAvidin ELISA, differences were observed between the groups <6 days and non-reactive control (*), 7-13 days and syphilis control (*), and >14 days and syphilis control (**). In the indirect ELISA, there was significant reactivity between the groups <6 days and non-reactive control (*), and 7-13 days and non-reactive control (**). For IgM peptides, significant reactivity of the LipL49/3M peptide was observed in the NeutrAvidin and indirect tests between the group 7-13 days and non-reactive control (*). The LipL49/5M peptide showed statistically significant differences in the NeutrAvidin tests between the groups <6 days and syphilis control, 7-13 days and non-reactive control, and >14 days and syphilis control (**). In the indirect ELISA, significance was also detected between the 7-13 days and non-reactive control (**). These results indicate diagnostic potential at different stages of infection, including the possibility of a bplex test between leptospirosis and syphilis.

Keywords: ELISA NeutrAvidin; Indirect ELISA; IgG and IgM Epitopes.

3055 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Study of the Protective Potential of Ora-Pro-Nóbis Extract in Sepsis-Associated Liver Injury

DOS SANTOS, P.D.M.A.; TERRA, E.; CORDEIRO, A.D.F.; NASCIMENTO, A.L.R.; DA LUZ, C.S.; TAKEUTI, V.Y.; PEREIRA, L.M.M.; ALVES, R.F.; REIS, P.A.. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Sepsis is a global medical emergency recognized by the WHO, causing approximately 11 million deaths annually. It is characterized by an exacerbated inflammatory response that leads to metabolic reprogramming of immune cells, favoring glycolysis over oxidative phosphorylation and stimulating lipid synthesis, which may contribute to organ failure, especially in the liver. *Pereskia aculeata* Mill. (commonly known as Ora-Pro-Nóbis) is a cactus native to the Americas, widely used in traditional medicine for its anti-inflammatory, antibacterial, and lipid metabolism-modulating properties. This study evaluated the phytochemical profile of the crude aqueous extract of *P. aculeata* (EAPa) and its effect on liver injury associated with experimental sepsis. The extract was obtained by infusion of the leaves and analyzed by thin-layer chromatography (TLC), using iodine vapor, sulfuric vanillin, and NP-PEG as revealing agents, indicating the presence of double-bonded compounds, terpenes, and flavonoids. Experimental sepsis was induced using the cecal ligation and puncture (CLP) model in male C57BL/6 mice, divided into three groups: SHAM (healthy control), CLP (negative control), and CLP+EAPa (25 mg/kg, orally). EAPa treatment was administered 1 hour before surgery and at 6, 24, and 48 hours post-surgery. All groups received Meropenem (10 mg/kg, subcutaneously) at the same time points. Livers were collected at 6 and 24 hours, perfused with PBS, fixed in 4% paraformaldehyde, and stained with Oil Red O for histological analysis. Plasma biochemical assays were performed at 6 and 24 hours. TLC revealed compounds with double bonds and polyphenols. EAPa treatment significantly increased survival and improved the clinical scores of septic animals. Biochemical and histological analyses showed relevant differences between the treated and CLP groups. These findings indicate the therapeutic potential of EAPa in protecting the liver during sepsis, although further studies are needed.

Keywords: Medicinal Plants; Sepsis; Infectious diseases.

3059 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

NK-CD8⁺ cells exhibit enhanced cytotoxicity in patients with severe COVID-19

SARMENTO, I.V.¹; LOPES, P.O.¹; DE MOURA, R.G.¹; DE CASTRO, C.H.D.F.¹; DE MIRANDA, P.H.¹; DE OLIVEIRA, A.L.¹; MILL, J.G.¹; FARDIN, J.M.¹; FARIA, A.M.C.²; COVRE, L.P.¹; AKBAR, A.³; GOMES, D.C.D.O.¹. 1. UFES, VITÓRIA - ES - BRASIL; 2. UFMG, BELO HORIZONTE - MG - BRASIL; 3. UCL, LONDON - REINO UNIDO.

COVID-19, caused by SARS-CoV-2, primarily affects the respiratory system and may progress to severe acute respiratory syndrome (SARS), characterized by intense inflammation, cytokine storm, and increased risk of death. This inflammatory environment can alter innate immune subpopulations, particularly NK cells, including CD3⁺CD56⁺CD8⁺ subsets, which are associated with antiviral responses in infections such as CMV and HIV. Evidence suggests that their hyperactivation may contribute both to viral control and to immunopathogenesis. This study aimed to phenotypically characterize this subpopulation in patients with mild and severe COVID-19, assessing potential implications for clinical outcomes. Peripheral blood samples collected between June 2020 and January 2021 were analyzed by flow cytometry (Cytoflex LX) using four panels (surface, intracellular, and phosphoflow). Markers of differentiation (CD57, KLRG1), cytotoxicity (granzyme B, perforin, NKG2D), and DNA damage (γH2AX) were evaluated, along with serum quantification of IL-6, IL-8, IL-10, and MCP-1 by ELISA. Although no significant differences in CD8 expression were observed, CD3⁺CD56⁺CD8⁺ cells in severe cases showed a trend toward increased CD57 expression—associated with terminal differentiation—and upregulation of granzyme B, perforin, and NKG2D, indicating an intensified cytotoxic profile. Additionally, elevated γH2AX levels were detected, suggesting inflammation-induced cellular stress. CD16 analysis revealed differences only in CD3⁺CD56⁺CD8⁺ cells, indicating distinct functional subtypes, while KLRG1 expression remained unchanged, suggesting regulation by other mechanisms. Both pro- and anti-inflammatory cytokines were elevated in severe cases, reflecting a microenvironment that promotes excessive NK cell activation and may contribute to the immunopathogenesis of severe COVID-19.

Keywords: NK cells; Cytotoxicity; COVID-19.

3064 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

B cell perturbations in HIV controllers compared to antiretroviral treated people

ABRAHÃO, M.M.¹; SOUZA, L.R.¹; DE LIMA, A.C.S.¹; CAETANO, D.G.¹; BELLO, G.¹; DE AZEVEDO, S.S.D.¹; HOAGLAND, B.²; VILLELA, L.M.²; CARDOSO, S.W.²; GRINSZTEJN, B.²; MORGADO, M.G.¹; GUIMARÃES, M.L.¹; CÔRTEZ, F.H.¹. 1. LABORATÓRIO DE AIDS E IMUNOLOGIA MOLECULAR, INSTITUTO OSWALDO CRUZ (IOC), FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. LABORATÓRIO DE PESQUISA CLÍNICA EM IST E AIDS, INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS (INI), FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

HIV-1 infection is accompanied by massive bystander activation, impairing many components of the immune system, including T and B cells. HIV controllers (HIC) are a rare group of people living with HIV (PLWH) who naturally control viral replication. However, some HICs experience disease progression after long-term control. This study compared the frequency of circulating Tfh cells (cTfh) and subsets of B cells between HIC and ART-treated PLWH (ART).

Peripheral blood mononuclear cells (PBMCs) were collected from 18 HICs, including 10 elite controllers (ECs) and 8 viremic controllers (VCs). The ART group included 7 PLWH with suppressed viral load. All participants were followed at the Instituto Nacional de Infectologia Evandro Chagas. PBMCs were stained with anti-CD3, CD4, CD45RO, CXCR5, and PD-1 for cTfh cells, and anti-CD19, CD3, CD10, CD27, CD38, and IgD for B cell subsets. All samples were stained with FVS450 to exclude dead cells. Samples were acquired using a FACS Aria flow cytometer and analyzed in FlowJo. Flow cytometry data were analyzed using ANOVA and Kruskal–Wallis with post hoc pairwise tests.

We found a similar frequency of cTfh and total B cells among the groups. However, when we analyzed the B cells subsets, we observed a higher frequency of plasmablasts ($p=0.0203$), tissue-like memory (TLM) B cells ($p=0.0423$), CD21neg MZ-like B cells ($p=0.0208$) and CD21neg Naïve B cells ($p=0.0211$) in VC compared to the ART group. VC also presented a higher frequency of TLM B ($p=0.0423$) cells and CD21neg Naïve B cells ($p=0.0208$) compared to EC group. No significant differences in B cell subsets were observed between the EC and ART groups.

The EC group did not show significant alterations in B cell subset frequencies compared to the ART group. We observed the opposite when comparing VC and ART groups, reinforcing that the use of ART in HICs with low levels of viral replication could reduce some of the immune disturbances during HIV infection.

Keywords: B cell;HIV;HIV CONTROLLERS.

3070 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Tetramethylpyrazine: A Potential Antifibrotic Agent in Experimental Mansonic Schistosomiasis

DOS SANTOS, A.C.C.; FIGUEIREDO-VANZAN, D.; BENTES, J.; MOTTA, J.M.; MATA-SANTOS, H.A.; PYRRHO, A.D.S.; CASTELO-BRANCO, M.T.L.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Hepatic fibrosis resulting from human mansonic schistosomiasis is one of the main causes of morbidity in helminthiasis. It's caused by the deposition of helminth eggs in the liver tissue, followed by a periovular granulomatous inflammatory reaction. Schistosomiasis is a neglected disease that affects 250 million people worldwide. In this context, praziquantel (PZQ), the antihelminthic drug of choice for helminth elimination recommended by the World Health Organization (WHO), requires additional treatment to reverse this sequelae. In this study, we evaluated the action of tetramethylpyrazine (TMP), a natural product with demonstrated anti-inflammatory, antifibrotic, and hepatoprotective effects in other experimental models in attenuating hepatic fibrosis caused by mansonic schistosomiasis. The data obtained from *in vivo* experimental study showed that the treatment with TMP reduced the size of hepatic granulomas, as revealed by histological analysis. *In vitro* experiments showed that TMP, in a dose-dependent manner, increased the levels of the anti-inflammatory cytokine IL-10 and downregulated the levels of the pro-fibrotic cytokine TGF- β 1. Moreover, the immunofluorescence analysis demonstrated that TMP in a dose-dependent manner inhibited collagen expression. Taken together, our results indicate that TMP exhibited an anti-inflammatory and antifibrotic effect in the model of hepatic fibrosis resulting from *S. mansoni* infection. The proposed experimental protocol was authorized by The Ethics Committee on the Use of Animals (CEUA) in Scientific Experimentation at the Health Sciences Center of Federal University of Rio de Janeiro registered with the National Council for Animal Experimentation Control (CONCEA). Approval No.002/18

Keywords: Tetramethylpyrazine;Schistosoma mansoni;Hepatic fibrosis.

3079 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Dormant *Cryptococcus neoformans* yeast remain hidden in a murine infection model

DE SOUZA, R.L.L.¹; TRINDADE, G.B.¹; BASSI, V.²; NETO, J.B.²; DE CASTRO, R.J.A.³; MARINA, C.L.F.³; SILVA, J.S.⁴; BOCCA, A.L.³. 1. RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. UNIVERSITY OF BRASÍLIA, BRASÍLIA - DF - BRASIL; 4. FIOCRUZ-BI-INSTITUTIONAL TRANSLATIONAL MEDICINE PROJECT, RIBEIRÃO PRETO - SP - BRASIL.

Several microorganisms, such as *Cryptococcus neoformans*, have the ability to remain in a dormant state, an adaptation in which cells reduce their metabolism, transcription, and translation to remain alive in suboptimal conditions for growth, with the possibility of resuming growth when favorable conditions arise. In this context, the main objective of our work is to understand which factors of the host immune response can prevent or trigger the reactivation of the human pathogenic fungus *C. neoformans* in its dormant state. To this end, we employed a protocol to induce in vitro dormancy of *C. neoformans*, allowing the generation of many viable but non-culturable (VBNC) yeasts. Our group developed a murine infection protocol to induce a latent infection using *C. neoformans* yeasts. Our preliminary results showed more remarkable survival in animals infected with VBNC. Furthermore, no fungal recovery occurred after pulmonary tissue culture when assessing colony-forming units. However, after immunosuppression of the animals, a high fungal load was recovered from the lungs and brains of the mice, demonstrating a viable infection, which, however, did not result in fatal consequences for the host. TLR9-deficient mice, as well as bone marrow-derived macrophages from animals that do not express TLR9, showed greater reactivation of dormant fungi compared to wild-type animals, even in the absence of immunosuppression. This indicates the participation of this molecule in maintaining the dormancy of *C. neoformans*. Additionally, flow cytometry analyses demonstrated a lower presence of monocyte-derived dendritic cells in TLR9-deficient animals infected with the dormant fungus, suggesting the participation of this cell type in maintaining fungal dormancy. Our data suggest that the mouse immune system can inhibit the reactivation of the fungus in the VBNC form, thus protecting the animal from the damage caused by infection.

Keywords: *Cryptococcus*;reactivation;immune system.

3080 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Immunopathogenicity of *Aspergillus nidulans* Clinical Isolates

ROCHA, P.¹; DA GRAÇA-FILHO, R.V.²; PINZAN, C.F.²; SILVA, P.A.D.C.²; FUENTES, D.L.P.²; GOLDMAN, G.H.². 1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - MG - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Aspergillus species are responsible for more than two million human infections each year, with host colonization dependent on the intricate regulation, the synthesis, and the remodeling of essential fungal cell wall carbohydrates and various proteins. Invasive pulmonary aspergillosis (IA) impacts immunocompromised individuals or those with underlying, pre-existing lung conditions. *Aspergillus nidulans* infections are frequently associated with chronic granulomatous disease (CGD), a specific hereditary immunodeficiency characterized by impaired NADPH oxidase activity and significantly compromised reactive oxygen species (ROS) production. Despite this recognized association, the actual immunopathogenic diversity of distinct *A. nidulans* strains remains, regrettably, poorly understood. Objective: To characterize the specific immunopathogenic potential of various clinical isolates of *A. nidulans* and to explore their relevance within context of chronic granulomatous disease.

We evaluated a total of 24 distinct clinical isolates of *A. nidulans* by infecting bone marrow-derived macrophages (BMDMs) at a specific multiplicity of infection (MOI) of 10. After 24 hours, we assessed their pro-inflammatory cytokine production (including TNF, IL-6, IL-1 β , and IL-10) and also determined conidial survival. Strains 82/060, 77/054, and CM 8754 notably induced significantly higher cytokine levels and exhibited enhanced survival within the infected macrophages. Subsequently, we will analyze the *in vivo* virulence of these selected strains in a murine Aspergillosis model via intranasal inoculation (with 5×10^8 conidia), using *A. fumigatus* (A1163) as a reliable positive control.

Our findings reveal significant heterogeneity in the immunopathogenicity of *A. nidulans* clinical isolates, with certain strains demonstrating virulence comparable to *A. fumigatus*. We plan to investigate the underlying mechanisms explaining the prevalence of *A. nidulans* infections in a murine CGD model.

Keywords: *Aspergillus nidulans*;Chronic granulomatous disease;infection.

3087 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Detection of Pure Neural Leprosy by *Mycobacterium leprae* antigens using Protein A/G-based ELISAVALLE, G.P.C.¹; ROCHA, A.C.¹; LIMA, A.A.L.¹; DIAS, P.M.¹; PINHEIRO, R.O.²; TEIXEIRA, H.C.¹. 1. UFJF - JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. FIOCRUZ-RJ, RIO DE JANEIRO - RJ - BRASIL.

Pure neural leprosy (PNL) is a form of leprosy whose principal manifestation is peripheral nerve involvement. Diagnosis is complex, as there are no clear dermatological signs, and identifying the bacillus in skin smears or even nerve biopsies can be tough. Recent research is seeking to develop more sensitive and specific serological tests for PNL. Here we investigated serum IgG reactivity against six *Mycobacterium leprae* specific antigens: NDO-HSA, a conjugate formed by natural octyl disaccharide bound to human serum albumin; LID-1, the fusion protein product of the ml0405 and ml2331 genes; and NDO-LID, the combination of LID-1 and NDO. Three recombinant proteins from *M. leprae*, ML2331, ML0405 and ML2055 were also included in this study. Sera from PNL, paucibacillary (PB) and multibacillary (MB) leprosy patients, and sera from two control groups, comprising other neuropathies (ON) and operational control (OP) (n=15/group) were assessed for the presence of antigen-specific antibodies by an indirect enzyme-linked immunosorbent assay (ELISA) based on protein A/G, instead of secondary antibodies. Proteins A and G are molecules that bind to the Fc portion of IgG from many mammalian species. Diagnostic accuracy of each ELISA was evaluated by receiver operating characteristic (ROC) curve analysis. Our results reveal an increase in antibodies against LID-1, NDO-LID, NDO-HSA and ML0405 in both PNL and MB patients, suggesting that these antigens are suitable for detecting PNL and MB leprosy. In contrast, ML2331 and ML2055 only detected increased antibodies in the MB group. The detection of anti-*M. leprae* antibodies in PB patients showed low values, such as those observed in the ON and CO control groups. Our findings demonstrate that ELISA based on protein A/G using LID-1, NDO-LID, NDO-HSA and ML0405 antigens may represent a useful tool to the serodiagnosis of PNL patients and to differentiate MB from PB leprosy.

Keywords: Leprosy;Diagnosis;Recombinant proteins.

3090 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

In vitro and in vivo models for the study of the immune response against *C. diphtheriae* extracellular vesiclesSOUZA, L.; MUNIZ, L.M.C.; DE AMORIM, L.H.; VALDEZ, A.F.; BONILLA, J.J.A.; PONTES, J.K.; ALLONSO, D.; FIGUEIREDO, R.T.; NIMRICHTER, L.; OLSEN, P.; MOREIRA, L.O.. UFRJ, RJ - RJ - BRASIL.

Corynebacterium diphtheriae (Cdi) is the causative agent of diphtheria, a respiratory disease. Its main virulence factor is the diphtheria toxin (DT), the protein target of vaccination. Although vaccination has significantly reduced disease incidence, recent studies have reported an increase in Cdi isolation worldwide. Moreover, mutations in the DT and invasive infections caused by non-toxigenic strains highlight the limitations of the vaccine and emphasize the need to investigate other virulence mechanisms. In addition to DT, Cdi expresses other virulence factors such as adhesins, catalase and neuraminidase. Also, previous unpublished studies from our group have shown that Cdi produces extracellular vesicles (EVs) carrying virulence-associated components, including DT. This work aimed to assess the immune response against Cdi EVs using *in vitro* and *in vivo* models. For that, EVs isolated from toxinogenic (Tox) and non-toxinogenic (Atox) Cdi, were concentrated from the supernatant through ultrafiltration and ultracentrifugation, and EVs size and shape were analyzed by Zeta View instrument and transmission electron microscopy. For *in vivo* assays, Wild type, *Dectin-1*^{-/-} and *Tlr4*^{-/-} BMDMs were stimulated with different concentrations of isolated EVs. For *in vivo* assays, *Galleria mellonella* larvae and C57BL/6 mice were injected with live bacteria or EVs, and evaluated for survival and pathological score. Preliminary results showed EVs were able to induce the production of NO, TNF-α and IL-6 by BMDMs. However, *Dectin-1*^{-/-} BMDMs produced less TNF-α when stimulated with Atox-EVs, when compared to Tox-EVs, while in *Tlr4*^{-/-} BMDMs the production of cytokines was reduced after both EVs stimulation. Neither the bacteria nor the EVs significantly affected *G. mellonella* or mice survival or pathological score. Ongoing studies will show more detail aspects of *in vitro* and *in vivo* effects of Cdi EVs.

Keywords: EXTRACELLULAR VESICLES;DIPHThERIA TOXIN ;IMMUNITY .

3098 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Annexin A1 Regulates Inflammatory Response in Cutaneous Leishmaniasis Patients Caused by *L. braziliensis*

SANTOS, C.P.¹; NASCIMENTO, M.T.²; CARVALHO, E.M.³; DE CARVALHO, L.P.¹. 1. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. INSTITUTO GONÇALO MONIZ (IGM) – FIOCRUZ, SALVADOR - BA - BRASIL; 3. INSTITUTO NACIONAL DE CIÊNCIAS E TECNOLOGIA DE DOENÇAS TROPICAIS (INCT-DT), SALVADOR - BA - BRASIL.

Cutaneous leishmaniasis (CL) is characterized by the presence of ulcerated skin lesions. CL lesions present high levels of TNF, IL-6 and IL-1 β - associated with disease progression, increased frequency of lymphocytes and mononuclear phagocytes, and few parasites. Annexin A1 (ANXA1) is a calcium-dependent phospholipid-binding protein. It is induced by glucocorticoids and can regulate the production of inflammatory mediators. The anti-inflammatory effects of ANXA1 are largely mediated through its binding to the FPR2, resulting in the control of the inflammatory response. Ac2-26 is a mimetic peptide derived from the N-terminal portion of ANXA1. It reproduces many of the biological and anti-inflammatory actions of the full ANXA1 protein. To investigate the effects of ANXA1 in IL-1 β , TNF, IL-10 and IL-6 production by *L. braziliensis* (LB)-infected macrophages and in biopsies from patients with CL. Skin biopsies from patients and healthy subjects (HS) were cultured in presence or absence of rANXA1 for 48h. Monocyte-derived macrophages from HS were infected with LB (5:1) in presence or absence of Ac2-26 and cultured for 8 and 48h. Levels of ANXA1 and IL-1 β , TNF, IL-10 and IL-6 were determined in supernatants of all cultures by ELISA assay. We observed increased levels of IL-1 β , IL-10, TNF and IL-6 in biopsy supernatants from CL compared with HS. In addition, we observed that rANXA1 treated supernatants of biopsies from CL patients, decreased IL-1 β and IL-6 levels, but did not affect TNF or IL-10 production. LB-infected macrophages produced high levels of IL-1 β , IL-10, TNF and IL-6. Finally, we found that treatment with Ac2-26 of LB-infected macrophage cultures decreased IL-1 β levels without affecting the levels TNF, IL-10, IL-6 and parasite load. Our results suggest that therapy with Ac2-26 or rANXA1 downregulates IL-1 β and IL-6 in patient skin and downregulates IL-1 β production in infected-macrophages without affecting the ability of macrophages to kill LB.

Keywords: Cutaneous leishmaniasis; Annexin A1; Ac2-26.

3100 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Cellular Proliferation of PBMCs from Cattle Immunized with *Rhipicephalus microplus* Larval Extract encapsulated in NIBDAF Nanoadjuvant: A Prospective Comparative MTT Assay Analysis

PEIXOTO, L.L.T.D.¹; SOUZA, P.E.D.A.²; APONTE, P.F.C.³; DE PAULA, L.S.M.³; MONTAÑA, J.E.L.³; BONATTO, C.C.⁴; SILVA, L.P.⁵; PRATA, M.A.⁶; MOREIRA, L.D.S.²; PEREIRA, V.S.D.A.⁶; CAMPOS, M.M.⁶; MACHADO, M.A.⁶; FACURY, T.M.³; CARVALHO, W.A.⁶. 1. UNIPAC, JUIZ DE FORA - MG - BRASIL; 2. UFJF, JUIZ DE FORA - MG - BRASIL; 3. UFMG, BELO HORIZONTE - MG - BRASIL; 4. EMBRAPA GENETIC RESOURCES AND BIOTECHNOLOGY, BRASÍLIA - DF - BRASIL; 5. EMBRAPA GENETIC RESOURCES AND BIOTECHNOLOGY, BRASÍLIA - DF - BRASIL; 6. EMBRAPA DAIRY CATTLE, JUIZ DE FORA - MG - BRASIL.

The NIBDAF is a biocompatible immunomodulatory target-delivery system designed to enhance immune responses without inducing cytotoxicity (intellectual property protection in progress). The use of tick-derived immunogens encapsulated in NIBDAF is a promising strategy for modulating immune responses against *Rhipicephalus microplus*.

To evaluate the viability and proliferative profile of PBMCs from cattle immunized with *R. microplus* larval extract (EL), NIBDAF, or the combination of both (NIBDAF-EL), compared to saline control groups, using the MTT assay.

PBMCs were isolated from blood of immunized Holstein calves and stimulated in vitro with PMA (mitogen), EL and *R. microplus* saliva antigens (both 1:1000) or saline solution (baseline control). MTT assays were performed according kit instructions (ThermoScientific), and 550nm optical density (OD) values were quantified to estimate mitochondrial activity as a proxy for cell viability and proliferation. Data were analysed using one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$). PMA-stimulated PBMCs from the NIBDAF-EL group showed higher viability than the EL (mean OD: 319.8 vs. 276.5) and NIBDAF (mean OD: 364.1) groups, suggesting synergistic effects on cell activation. Under EL stimulation, the NIBDAF-EL group also demonstrated higher proliferation than the EL and saline controls (mean OD: 223.7 vs. 125.6 and 83.4, respectively; $p < 0.05$). The saline group showed basal viability, with no significant differences among immunized animals. Control animals had intermediate viability under all conditions.

In conclusion, immunization with NIBDAF-EL induced greater activation and viability in PBMCs, suggesting that the NIBDAF platform enhances the immunostimulatory capacity of larval antigens and holds promise for the development of anti-tick vaccines. Complementary studies on gene expression and cytokine profiles are ongoing to better characterize the immunomodulatory profile.

Keywords: *Rhipicephalus microplus*; Holstein calves; immunomodulatory.

3105 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Functional profile of $\gamma\delta$ T cells is associated with COVID-19 clinical severity and pathogenesis

CAZOTE, A.D.S.; MASCARENHAS, G.D.; PERAZZO, H.; GERALDO, K.M.; RIBEIRO, M.P.D.; DE MATOS, J.A.; DO BRASIL, P.E.A.A.; CARDOSO, S.W.; GRINSZTEJN, B.; VELOSO, V.G.; CASCABULHO, C.M.; GOULART, M.N.G.; DE SÁ, N.B.R.; DE ALMEIDA, D.V.; CÔRTEZ, F.H.; MORGADO, M.G.; GRIPP, C.B.W.G.. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Background: COVID-19 includes a broad spectrum of clinical manifestations, with uncontrolled inflammation contributing to disease severity. Understanding the nature of the immune response to COVID-19 pathogenesis is crucial for developing effective treatments. We aim to assess the role of gamma delta ($\gamma\delta$) T cells in this process by examining their potential *in vitro* responses and correlating them with the clinical severity of COVID-19 patients.

Methods: Forty-four COVID-19 inpatients admitted at the Hospital Center for COVID-19 Pandemic from the National Institute of Infectious Disease-FIOCRUZ, Brazil, were included in this study. Based on the WHO Clinical Progression Scale, patients were classified as moderate without oxygen support (MWO₂; n=15), moderate with oxygen support (MO₂; n=15), or severe disease (SD; n=14), requiring mechanical ventilation support. $\gamma\delta$ T cells were isolated from peripheral blood cells by magnetic beads and cultured under OKT3 antibody and K562 lineage cells for 16 hours. Cytokine production (TNF- α , IFN- γ , IL-17) and expression of TRAIL, CD27, CD107a, IL-23R, and IL-1R were evaluated in total $\gamma\delta$ T cells and V δ 1 and V δ 2 subsets by flow cytometry.

Results: MWO₂ patients had significantly higher frequencies of circulating V δ 1 $\gamma\delta$ T cells than MO₂ ones ($p<0.05$) and the median fluorescence intensity (MFI) for CD27 was significantly higher in MWO₂ patients, when compared with MO₂ and SD groups ($p<0.05$), for total $\gamma\delta$ T cells or V δ 1 and V δ 2 subsets. In addition, MWO₂ patients presented higher frequencies of IL-17⁺ and TRAIL⁺ V δ 2 cells, higher IL-1R MFI and lower IL-23R MFI in V δ 2 cells compared to other groups ($p<0.05$).

Conclusion: The higher frequency of V δ 1 $\gamma\delta$ T cells, the higher MFI observed to the memory-like receptor CD27, and the functional profile of V δ 2 cells in MWO₂ patients suggest an active role for these cells in a more effective immune response, limiting COVID-19 severity and favoring improved clinical outcomes.

Keywords: COVID-19; $\gamma\delta$ T cells;innate immunity.

3106 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Extracellular vesicles from human neutrophils modulate cellular functions and impact infection caused by *Leishmania amazonensis*

WACLAWIAK, I.¹; WERNECH, M.V.I.¹; RAMOS, C.D.L.¹; SILVA, C.A.A.²; COSTA, A.G.B.¹. 1. MICROBIOLOGIA PAULO DE GOES, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO, RIO DE JANEIRO - RJ - BRASIL.

In *Leishmania* infection, neutrophils are the first cells to migrate to the inoculation site, where they can either kill the parasite or exacerbate the infection. Extracellular vesicles (EVs), released by nearly all cells, including neutrophils, regulate various physiological processes, potentially affecting tissue homeostasis or spreading infectious agents. Neutrophils release EVs in response to inflammatory stimuli, playing immunomodulatory roles. While the role of neutrophils in *Leishmania* infection has been well studied, the impact of EVs released by these cells in response to the parasite remains underexplored.

This study aimed to characterize EVs released by human neutrophils stimulated by *Leishmania amazonensis* promastigotes and to examine their effects on neutrophil modulation *in vitro* and infection *in vivo*. Neutrophils isolated from healthy individuals were stimulated with *L. amazonensis* promastigotes, and small extracellular vesicles (sEVs) were obtained by ultracentrifugation. Nanoparticle tracking analysis (NTA) and electron microscopy showed that sEVs formed a homogeneous population of approximately 150 nm vesicles, with parasite stimulation increasing vesicle production.

The vesicles were found to be non-toxic to neutrophils and did not alter parasite phagocytosis. However, the stimulated sEV fraction led to increased NET production, independent of ROS and dependent on PAD4. Both sEVctrl and sEVLa reduced IL-8 production by neutrophils, but sEVLa-treated neutrophils showed enhanced migration toward this chemokine. *In vivo*, sEVs worsened ear lesions caused by infection, while footpad infection resulted in smaller lesions with sEVLa, suggesting that the effects of these vesicles may vary depending on the infection site.

Future studies will focus on characterizing the vesicle content and exploring the complex mechanisms by which they influence infection outcomes.

Keywords: Extracellular Vesicles;Leishmania amazonensis;Neutrophils.

3124 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

EXPRESSION OF NKILA AND PACER LONG NONCODING RNAs IN MACROPHAGES INFECTED WITH LEISHMANIA INFANTUM.

LIMA, C.N.C.; ZANATTA, J.M.; ELEUTÉRIO, B.P.; TEIXEIRA, C.A.; KATO, K.T.; BAHIA, I.A.F.; DA FONSECA, D.L.M.; CRUZ, L.O.R.; 22/00291-4, S.M.M.F.. USP, SÃO PAULO - SP - BRASIL.

Leishmaniasis, caused by the protozoan parasite *Leishmania infantum*, modulates host macrophage immune responses to ensure parasite survival. Long non-coding RNAs (lncRNAs), such as NKILA (NF- κ B Interacting lncRNA) and PACER (p50-Associated COX-2 Extragenic RNA), are critical regulators of inflammation and NF- κ B signaling. The aim is investigate the expression of lncRNAs NKILA and PACER, and genes related to NF- κ B activation in THP-1 macrophages infected with *Leishmania infantum*. We designed primers targeting different regions of the mature lncRNA, and found that NKILA expression increased significantly with LPS stimuli at 4 and 24h, but not modified under infection. The expression of PACER was reduced in macrophages infected with *L. infantum* at 4h, but increased at 24h, suggesting a time-dependent regulation. LPS stimulation induced a significant increase in PACER expression at both 4h and 24h. The transcriptome and RT-qPCR analysis in THP-1 macrophages infected with *L. infantum* showed that genes related to NF- κ B, such as NOS-2, decreased during infection, while Cox-2 increased only at 24 h, and IL-1 β and Rel-A showed variations during infection. Meanwhile, LPS stimulation increased IL-1 β , Rel-A, Cox-2, and NOS-2 genes, which showed variations at certain times. Also, Rel-B, NF- κ B 1, NF- κ BIE and NF- κ B 2 expression was higher in the transcriptome analysis of macrophages infected with *L. infantum* at 24h. Our data suggest that *L. infantum* infection and LPS stimulation modulate the expression of NKILA and PACER in distinct ways. Analysis of the reduction and overexpression of NKILA and PACER during *L. infantum* infection and CRISPR-Cas9 knockout strategy for NKILA and PACER may help us understand their function and other genes induced by NF- κ B.

Keywords: Leishmania;Long non-coding RNAs;NF- κ B.

3129 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

CHARACTERIZATION OF A VACUOLAR ALPHA-MANNOSIDASE FROM *Paracoccidioides brasiliensis* AND EVALUATION OF ITS POTENTIAL AS A THERAPEUTIC TARGET IN EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS

NERES, P.C.C.; GONÇALVES, T.A.R.P.; CASTELO, A.P.. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Introduction:

Paracoccidioides brasiliensis is a thermomimorphic fungus and the causative agent of paracoccidioidomycosis (PCM), with Brazil accounting for approximately 85% of cases. In the virulent Pb18 strain, we identified an 85-90-kDa vacuolar alpha-mannosidase in the culture supernatant, exhibiting enzymatic activity and increased expression during the yeast phase—features associated with virulence. Its extracellular presence suggests release into the host, highlighting its potential as an immunological and therapeutic target.

Methods and Results

Western blotting and ELISA techniques demonstrated that recombinant AMS (rAMS) binds nonspecifically to polyclonal and monoclonal antibodies, raising the hypothesis that it possesses lectin-like properties, with an affinity for glycosylated portions of immunoglobulins. Reinforcing this, AMS binds to highly glycosylated proteins, such as fetuin and asialofetuin, as well as to mannose, which blocks these non-specific interactions. In addition to these characterizations, in vitro assays showed that AMS stimulates the production of inflammatory cytokines (IL-6 and TNF) by bone marrow-derived macrophages and promotes spleen cell proliferation in a dose-dependent manner. In an in vivo model of experimental PCM, induced by intranasal and intravenous infection with *P. brasiliensis*, subcutaneous and intranasal treatment with rAMS and its messenger RNA resulted in a reduction in pulmonary fungal burden, although without a significant impact on tissue inflammation.

Conclusion

Functionally, binding assays suggest that AMS may possess a carbohydrate recognition domain, based on its high-affinity interaction with glycosylated molecules such as fetuin and antibodies, indicating a putative lectin-like function. Therapeutically, rAMS or AMS mRNA reduces fungal burden in experimental PCM without significantly affecting inflammation, supporting the potential of novel strategies over conventional treatments.

Financial Support: CNPq and FAPESP.

Keywords: Paracoccidioidomycosis;Alpha-Mannosidase;Therapy.

3139 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

MicroRNAs miR-372 and miR-373 participate in the regulation of HIF1A and metabolic shift in macrophages infected with *Leishmania infantum*.

ELEUTÉRIO, B.P.¹; ZANATTA, J.M.¹; TEIXEIRA, C.A.¹; LIMA, C.N.C.¹; DE OLIVEIRA, V.¹; BAHIA, I.A.F.¹; LÓPEZ-GONZÁLEZ, M.Á.²; BARBAS, C.²; 22/00291-4, S.M.M.F.¹. 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. CENTRE OF METABOLOMICS AND BIOANALYSIS, MADRID - ESPANHA.

HIF-1 α (hypoxia-inducible factor-1 α) is a transcription factor involved in the expression of genes that participate in glycolytic metabolism and the lactate production pathway. In macrophages, HIF-1 α is activated in response to PAMPs, such as LPS, leading to a reprogramming of carbohydrate and lipid metabolism and an induction of inflammation. MicroRNAs are small non-coding RNAs that regulate gene expression by pairing their seed (6-9 nt) with the complementary 3' UTR sequence of the target mRNA, promoting degradation or inhibition of mRNA translation. Here, we aimed to evaluate the function of miR-372 and miR-373 in the regulation of HIF1A (subunit of HIF1 α), and its network of target genes and their impact in the metabolic shift in macrophages infected with *L. infantum*. With the use of RT-qPCR and flow cytometry, we analyzed the expression of miR-372/373, HIF1A and two target genes (GLUT1 and LDHA), and the rate of infection, respectively. We observed an increase in the expression of miR-372 and miR-373 and decreased the expression of HIF1A at 4 and 24 hours of infection, in THP-1 derived macrophages infected compared to uninfected or LPS stimuli. Furthermore, we showed an increase in the expression of pre-miR-372 and pre-miR-373 at 1 and 2 hours of infection, which can be correlated with reduction in HIF1A. The inhibition of miR-372 and miR-373 diminished the rate of infection. Using CE-MS, we analyzed the metabolic shift with the inhibition and negative control of the microRNAs. We found an alteration in glucose related pathways, such as glycolysis and pentose-phosphate in a way that when comparing miR-inhibited to negative control groups, glycolysis performed higher in the enrichment analysis than the pentose-phosphate pathway, meaning that it can be more active with the inhibition of miR-372 and miR-373. Our data suggest the regulation of HIF1A by miR-372 and miR-373 and impacting infection.

Keywords: HIF1A;Leishmania;miRNA.

3145 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

EFFECT OF IMMUNOSENESCENCE BY DENDRITIC CELLS DURING CHRONIC HTLV-1 INFECTION

CONSTANCIO, C.D.S.¹; CAMPOS, M.C.S.¹; DA SILVA, M.T.T.²; LIMA, M.A.²; LEITE, A.C.C.B.²; BOZZA, M.T.¹; COBOS, E.V.M.¹; DE LIMA, J.E.N.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. LABORATÓRIO DE PESQUISA CLÍNICA EM NEUROINFECÇÕES, INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS, FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Immunosenescence changes the cellular profile and functionality of the immune system during aging. Beyond age, certain factors contribute to immunosenescence, including chronic infections. The human T-cell lymphotropic virus type 1 (HTLV-1) is the etiologic agent of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM), a chronic inflammatory and demyelinating disease. This disease can be related to a premature cell senescence process. Among other immune cells, plasmacytoid and conventional dendritic cells (pDCs, cDCs) are present in reduced numbers and altered functionality in the elderly. Since DCs are essential for antiviral responses, we aimed to explore how DCs subsets change in HTLV-1 patients, and their impact on disease progression. **Methods and Results:** PBMCs from adult (29-59 years old) and aged (60-84 years old) asymptomatic carriers (AC), HAM patients, and uninfected donors (NI) were isolated and cultured with or without R848, a TLR7/8 ligand. Then, circulating DCs were characterized by flow cytometry. In elderly infected donors, an increased frequency of cDCs was detected compared to adult patients. This group of patients also showed reduced expression of CD86 in pDCs. In contrast, expression of the Fc γ RI (CD64) was increased compared to HTLV-1 infected adults. Additionally, we analyzed soluble factors in serum samples from HTLV-1 patients, and observed that IL-18 and Syndecan were markedly reduced in HTLV-1-infected adults compared to NI. **Conclusion:** These preliminary results suggest that both age and infection cause alterations in the DCs subsets of HTLV-1 patients, especially in regard to Fc γ R and costimulatory molecules. Further analysis of the functional implications of these changes are warranted. Moreover, the lower levels of circulating Syndecan and IL-18 may be linked to synaptic disfunction and reduced IFN- γ production in HTLV-1 adult patients, which could contribute to disease progression.

Keywords: Immunosenescence;HTLV-1;Dendritic cells.

3166 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Macrophage migration inhibitory factor (MIF) as a regulator of barrier function and disease tolerance in the intestinal mucosa

ROSA, S.G.; FERREIRA, L.D.S.; TEIXEIRA, G.A.; GAMA, A.M.D.S.; FERREIRA, D.C.; NAZIOBERTO DUDA DE FARIAS, J.; DE SOUZA, H.S.P.; VIDAL, V.M.; BOZZA, M.T.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Macrophage migration inhibitory factor (MIF) is a pleiotropic pro-inflammatory cytokine highly expressed in the gastrointestinal tract. Our group demonstrated that MIF-deficient (*Mif*^{-/-}) mice shelter a distinct gut microbiota characterized by high species diversity, dysbiosis-associated signatures, and enrichment in pathobionts/pathogens. Notably, transfer of this microbiota to inflammation-prone *Il10*^{-/-} recipients triggers acute and lethal colitis. To investigate how *Mif*^{-/-} hosts tolerate this potentially pathogenic microbial community without overt disease, we hypothesized that *Mif*^{-/-} mice display enhanced colonic mucosal barrier function. To test this, colon samples from *Mif*^{-/-} and wild-type mice were analysed under steady state conditions (CEUA 040/22). Histopathology (H&E) revealed no abnormalities, and inflammatory cytokines (*Tnfa*, *Il6*) were comparably expressed, confirming the absence of subclinical inflammation in *Mif*^{-/-} hosts. Mucus production, evaluated via PAS staining and *Muc2* expression, was similar between genotypes. Notably, flow cytometry and RT-qPCR demonstrated that *Mif*^{-/-} mice exhibit a higher frequency of EpCAM⁺ epithelial cells and a unique transcriptional profile, marked by upregulation of *Il22*, *Il17a*, *Epi-regulin*, *Il33* and *Tl1a* alongside downregulation of *Il5*, *Il13*, and *Relmb* - revealing a homeostatic shift toward Th17- over Th2-type responses. Given IL-22's pivotal role in barrier regulation, *Mif*^{-/-} mice were treated with neutralizing α -IL-22 antibodies or isotype control to disrupt endogenous tolerance. However, no disease onset or weight loss was observed, meaning that IL-22 may not be central to this phenomenon. Generation of a double-deficient *Mif*^{-/-} *Il22*^{-/-} strain is in progress. The low expression of antimicrobial peptides, such as *Relmb*, suggests a mechanism by which MIF shapes microbial composition. These findings indicate novel roles for MIF in regulating barrier function, intestinal immunity and microbiota selection.

Keywords: Mucosal Immunology;Microbiota;Inflammation.

3167 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

L. braziliensis exosomes induce celular and humoral immune response

PEIXOTO, F.D.C.; GUIMARÃES, J.M.C.S.; CARVALHO, A.M.; CARVALHO, E.M.; DE CARVALHO, L.P.. FIOCRUZ, SALVADOR - BA - BRASIL.

Leishmania exosomes (Evs) are composed by several proteins with signaling activity. It has been reported that these vesicles actively manipulate host signaling and immune cell functions, due to the enrichment by *Leishmania* exosomes of virulence factors. Studies demonstrate that *L. donovani* exosomes downregulate IFN- γ and inhibit the expression of microbicidal molecules such as TNF and NO, creating a microenvironment that favors parasite proliferation. Regarding *L. braziliensis* exosomes, it has been shown that priming macrophages before infection, increases secretion of both IL-6 and IL-1 β . For this study, we hypothesized that stimuli with *L. braziliensis* exosomes isolated from different clinical forms would induce distinct immune response *in vitro*. **Methods and Results:** Peripheral blood mononuclear cells (PBMC) from cutaneous leishmaniasis (CL) patients were stimulated with *L. braziliensis* Evs obtained from strains causing 3 different clinical forms of leishmaniasis; CL, mucosal (ML) or disseminated (DL), plus Soluble *Leishmania* antigen (SLA) or not, and cells were cultured for 72 hours. We observed higher secretion of IL-1 β , IL-10, IFN- γ and GRZ-B by cells stimulated with Evs from all strains, compared to basal level. Interestingly, cells stimulated with Ev CL showed higher levels of IFN- γ and IL-1 β than the ones with Ev ML. Furthermore, Ev ML regulated SLA- induced IFN- γ production, but increases levels of TNF and IL-1 β in costimulated cultures. Also, stimulation of PBMC with Ev CL increases CD4⁺CD25⁺ frequency, both compared to SLA and EV ML stimuli. We also evaluated the presence of Ev-specific IgG in the serum of CL patients, which elucidated a potential new diagnostic test reaching up to 95% Sensitivity. In addition, patients who failed therapy presents higher levels of IgG α Ev LC than those who cured. These data suggest a major role of Evs in CL pathogenesis, as well as a possible marker for therapy failure and a novel target for diagnostics.

Keywords: *Leishmania braziliensis*;exosomes;Cutaneous leishmaniasis.

3169 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Deficiency in macrophage migration inhibitory factor (MIF) promotes immune reprogramming in the intestinal mucosa

FERREIRA, L.D.S.; ROSA, S.G.; TEIXEIRA, G.A.; GAMA, A.M.D.S.; DE SOUZA, H.S.P.; VIDAL, V.M.; BOZZA, M.T.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Macrophage migration inhibitory factor (MIF) is a pleiotropic protein with pro-inflammatory and immunomodulatory functions. MIF is abundantly detected along the gastrointestinal tract. In experimental models of intestinal inflammation, MIF-deficient mice (*Mif*^{-/-}) are protected against disease, indicating that MIF exacerbates inflammation. Our group found that conventionally raised *Mif*^{-/-} mice exhibit a distinct fecal microbiota compared to wild-type (WT) controls, characterized by signatures typical of dysbiosis and enrichment in pathobionts/pathogens. This microbiota is sufficient to induce lethal colitis when transferred to *Il10*^{-/-} recipients, despite the absence of clinical signs in *Mif*^{-/-} donors - suggesting that disease tolerance mechanisms operate in these animals. Our group has been investigating disease tolerance mechanisms in the colon, yet little is known about the small intestine. Here, we hypothesized that *Mif*^{-/-} mice exhibit a more efficient intestinal mucosal barrier function in steady state. To test this, jejunum sections from WT and *Mif*^{-/-} mice in homeostasis (CEUA-040/22) were collected for histology, flow cytometry to assess epithelial proliferation, and qRT-PCR to assess barrier, inflammatory, and regulatory genes. Histological architecture remained intact with comparable expression of inflammatory mediators (*Tnfa*, *Il6*, *Il33*), mucus components (*Muc2*, *Tff3*), antimicrobial peptides (*Reg3b*, *Relmb*), and regulatory cytokines (*Il10*, *Tgfb*). However, *Mif*^{-/-} mice showed increased EpCAM⁺ epithelial cell frequency, upregulated Th2-associated cytokines (*Il4*, *Il13*), and decreased junctional protein expression (*Occludin*, *Cadherin1*). These findings suggest that MIF acts as both a negative regulator and a promoter of intestinal barrier components, extending its role beyond inflammation. Ongoing intestinal permeability assays and immunophenotyping of CD4⁺ T cells and ILCs will further characterize the functional and cellular basis of this phenotype. Keywords: Disease tolerance; Intestinal mucosa; Inflammation.

3192 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Parasite Load and Host Gene Expression Assessed by qPCR as a Diagnostic Tool for Leishmaniasis Caused by *Leishmania braziliensis*

DE OLIVEIRA, F.D.A.P.¹; MAGALHÃES, A.S.²; CARVALHO, E.M.²; DE CARVALHO, L.P.². 1. UFBA-UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. FIOCRUZ- FUNDAÇÃO OSWALDO CRUZ, SALVADOR - BA - BRASIL.

Introduction: Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is characterized by intense inflammation and few parasites. There is no gold standard for CL diagnosis and due to the low amounts of parasites in lesion, a direct diagnosis has been challenging. High levels of IL-1b and Granzyme B have been associated with poor clinical outcome in CL. Objectives: To determine parasite load, and IL-1b and Granzyme B genes expression in biopsy samples from CL patients, using qPCR. Methodology: DNA and mRNA samples from 34 CL were obtained. A qPCR assay was developed to quantify parasite load using the kDNA3 sequence as the target for detecting *L. braziliensis* and human 18S rRNA as a reference gene for sample normalization. IL-1b and Granzyme B relative genes expression were determined by using b-actin as a housekeeping gene Results: The qPCR technique proved to be sensitive (100%), allowing the detection and quantification of parasites even in samples with a considerably low parasite load. Parasite quantification by qPCR showed a strong positive correlation with the number of amastigotes observed through immunohistochemistry, validating the method. All samples were positive for IL-1b and Granzyme B. Positive correlation between parasite and IL-1b transcripts were observed. Conclusion: Our study demonstrates that parasite load quantification by qPCR is a valuable and sensitive tool for CL diagnosis. Ongoing studies are being carried out to investigate whether parasite load, IL-1b and Granzyme B gene expression may serve as a prognosis biomarker in CL. Keywords: Cutaneous leishmaniasis; qPCR diagnosis; Inflammatory biomarkers.

3209 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

IFN- γ and TNF do not contribute to IL-1 β production in cutaneous leishmaniasis due to *Leishmania braziliensis* infection.

DOS SANTOS, L.D.R.¹; PEIXOTO, F.D.C.¹; GUIMARÃES, J.M.C.S.²; CARVALHO, E.M.¹; DE CARVALHO, L.P.¹. 1. FIOCRUZ - BAHIA, SALVADOR - BA - BRASIL; 2. FIOCRUZ BAHIA, SALVADOR - BA - BRASIL.

Introduction. *Leishmania braziliensis* infection may lead to high inflammation, where IL-1 β production is associated with tissue damage and skin ulcer development. IFN- γ is the main cytokine that activates macrophages to kill *Leishmania* and TNF has been associated with poor longer time to heal and therapeutic failure. However, the contribution of IFN- γ and TNF to IL-1 β production in cutaneous leishmaniasis (CL) is not known. **Objectives.** To investigate the role of IFN- γ and TNF on IL-1 β production in CL. **Methodology:** Peripheral blood mononuclear cells from CL patients were obtained. *In vitro* stimulation with soluble *Leishmania* antigen (SLA) in presence or absence of monoclonal antibodies anti-IFN- γ and anti-TNF were performed. Cytokine concentrations were determined by ELISA on culture supernatants **Results:** High levels of SLA-induced IFN, TNF and IL-1 β were observed. Neither IFN- γ nor TNF levels correlated with IL-1 β levels. Neutralization of IFN- γ enhanced SLA-induced IL-1 β production. Neutralization of TNF did not alter IFN- γ levels. **Conclusions.** Our data show that IFN- γ and TNF do not contribute to IL-1 β production. In contrast, IFN- γ does prevent the production of high levels of IL-1 β . These data strengthen the protective role of IFN- γ in CL

Keywords: Leishmania;Immunology;IFN- γ .

3219 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Differential cell-mediated immune responses in individuals seropositive for LID-1 and PGL-1 - *Mycobacterium leprae* antigens

FRAGA, L.A.D.O.¹; LEITE, H.M.²; ., P.H.F.M.³; OLIVEIRA, L.B.P.⁴; VIEIRA, M.⁵; PINHEIRO, M.⁶; VELOSO., C.⁷; MONTEIRO, D.⁸; BRANCO, A.C.⁹; ALMEIDA, A.⁷; NETO, J.B.⁷; SCHNEIDER, M.⁷; MARTINS-FILHO, O.A.¹⁰; LIMA, M.R.¹¹; BRITO, J.¹²; MAGUETA, E.¹³; FAIRLEY, J.K.¹⁴. 1. FEDERAL UNIVERSITY OF JUIZ DE FORA CAMPUS GOV. VALADARES..PMBQBM, GOVERNADOR VALADARES - MG - BRASIL; 2. UFJF GV PMBQBM, UNIVALE, GV - MG - BRASIL; 3. UFJF GV PMBQBM, UNIVALE, GEORGIA TECH INSTITUTE/ATLANTA, GV - MG - BRASIL; 4. UFJF GV PMBQBM, UNIVALE, UCSD-SAN DIEGO, GV - MG - BRASIL; 5. UFJF GV PMBQBM, UNIVALE, GV - MG - BRASIL; 6. UFJF GV, PMBQBM, UNIVALE, GV - MG - BRASIL; 7. UFJF GV, GV - MG - BRASIL; 8. UFJF GV, PMBQBM, GV - MG - BRASIL; 9. CREDENPES-SMS-GV, GV - MG - BRASIL; 10. CPQRR-FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 11. UNIVALE, GV - MG - BRASIL; 12. CPQRR-FIOCRUZ - MINAS, BELO HORIZONTE - MG - BRASIL; 13. UFJF, GV - MG - BRASIL; 14. EMORY UNIVERSITY/ATLANTA/USA, ATLANTA - ESTADOS UNIDOS DA AMERICA.

Introduction: Brazil ranks second in the world for the number of new leprosy cases annually. An active search is essential for early detection, interruption of the transmission chain, and preventing disabilities and the consequences of late diagnosis. Defining immunological biomarkers for detecting subclinical infection is a great strategy to achieve this goal. **Methods:** Serum samples collected from healthy persons in 4 municipalities of eastern/MG (n=1,315) were tested for antibodies (IgG) against *M. leprae* /LID1, a recombinant fusion protein. **Individuals positive for anti-LID1 and matched anti-LID1 negative controls were then enrolled in a longitudinal study. Samples were taken for anti-PGL1 testing with ML-flow/Bioclin and peripheral blood mononuclear cells (PBMC).** Cytokine and chemokine levels were measured using the CBA method (BD), after *in vitro* culture, stimulated by *M. leprae* antigen. Cytokine and chemokine levels were compared across four serological groups: LID1+/PGL1+, LID1+/PGL1-, LID1-/PGL1+, and LID1-/PGL1- to identify differences in inflammatory signatures. **Results: 79 individuals tested positive for anti-LID1 IgG, and 76 anti-LID1 controls were included in the analysis.** Out of the 79 anti-LID1+ individuals, 30 were also positive for PGL1+. Seventeen were positive for PGL1 and negative for LID1, and 57 were negative for both. Using ascendant cytokine/chemokine signature analysis, the LID1+/PGL1- showed the most pro-inflammatory profile, being high producers of IFN- γ , CXCL8, CXCL9, CXCL10, TNF, IL-2, and IL-6. The LID1-/PGL1+ were high producers of CXCL8, CCL5, CCL2, and IL-4, while the double-positive group showed high production of only CCL2. **Conclusion:** The serological profile associated with the most ML-stimulated pro-inflammatory biomarkers was notably the LID1+/PGL1-group, indicating a strong immune response to *M. leprae*. The follow-up of these individuals will provide more insights to evaluate the risk of developing the disease.CNPq/NIH/FAPEMIG.

Keywords: leprosy;cytokines/chemokines;LID-1, PGL-1.

3223 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The Role of MicroRNA-294 and NLRP12 in Modulating Macrophage Response to *Leishmania amazonensis*

TEIXEIRA, C.A.; ZANATTA, J.M.; ELEUTÉRIO, B.P.; LIMA, C.N.C.; KATO, K.T.; BAHIA, I.A.F.; 22/00291-4, S.M.M.F.. INSTITUTO DE CIÊNCIAS BIOMÉDICAS - USP, SÃO PAULO - SP - BRASIL.

Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*, transmitted to humans by sand flies. It can present in cutaneous, mucocutaneous, or visceral forms, depending on the parasite species and the host immune response. *Leishmania amazonensis*, relevant in Brazil, causes cutaneous leishmaniasis and primarily infects macrophages, surviving within the phagolysosome and modulating inflammatory pathways, such as inflammasome formation. One immune evasion mechanism involves microRNAs (miRNAs), small endogenous RNAs that inhibit gene expression. In mice, miR-294 is transcribed from an intronic region of the *Nlrp12* gene, which negatively regulates inflammasome activation and inflammation. This study aimed to investigate the role of NLRP12 and miR-294 in macrophage responses to *L. amazonensis* infection. In BALB/c mice, which are susceptible to infection, expression of NLRP12, premiR-294, and miR-294 increased at 4 h post-infection, followed by a decrease in NLRP12 and premiR-294 at 24 h, with no modulation by LPS. IL-1 β , IL-18, and NLRP3 were not modulated by infection, but IL-1 β and NLRP3 increased after LPS stimulation. In C57BL/6 mice, which are resistant to infection, miR-294 expression increased at 24 h, and IL-1 β increased only in response to LPS. Inhibition of miR-294 in infected C57BL/6 macrophages increased NLRP12 and IL-1 β expression and reduced the frequency of infected macrophages and the number of amastigotes per cell (MFI) at 4 h. Additionally, silencing CEBP β (a transcription factor for NLRP12) and RelA (a TLR-activated NF- κ B subunit) also reduced infection at 4 h and 24 h, suggesting that regulation of NLRP12 by miR-294, CEBP β , and RelA influences the host response.

Keywords: microRNA;Leishmaniasis;;macrophages;.

3227 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

ACUTE DENGUE VIRUS INFECTION REPROGRAMS T CELLS TOWARD A SENESCENT AND CYTOTOXIC PHENOTYPE

ZORDAN, J.S.N.; SARMENTO, I.V.; PIMENTA, B.C.; FARDIN, J.M.; VICENTE, C.R.; COVRE, L.P.; GOMES, D.C.D.O.. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL.

Dengue virus (DENV) infection is a systemic, dynamic disease with a broad clinical spectrum, ranging from asymptomatic to life-threatening presentations. The acute phase (days 1-5 post-symptom onset) is critical in determining clinical outcome. The intense inflammatory milieu, as observed in dengue, may reshape T cell differentiation and function, favoring the accumulation of senescent phenotypes. These cells are defined by the progressive loss of costimulatory receptors (CD27, CD28), enhanced cytotoxicity, expansion of terminal effector memory cells (EMRA) subsets, and impaired proliferative capacity. Here, we investigated hallmarks of immunosenescence in T cells during acute DENV infection. All procedures were approved by the Research Ethics Committee for Human Subjects under protocol number 5.994.874. Peripheral blood mononuclear cells (PBMCs) were isolated from patients and healthy controls, and subjected to multiparametric flow cytometry to evaluate cell viability; frequencies of CD3 $^{+}$, CD4 $^{+}$, and CD8 $^{+}$ T lymphocytes; naive and memory subsets (based on CD45RA and CD27 expression), and cytotoxic potential through granzyme B expression. Preliminary data reveal elevated granzyme B expression in CD8 $^{+}$ T cells from DENV-infected individuals compared to healthy controls, indicating an enhanced cytotoxic activity during acute infection. Additionally, patients exhibited a decreased frequency of naive CD8 $^{+}$ T cells (CD45RA $^{+}$ CD27 $^{+}$) and an expanded population of EMRA cells (CD45RA $^{+}$ CD27 $^{-}$). These findings suggest that acute DENV infection promotes a shift toward a cytotoxic, senescent-associated T cell phenotype, which may contribute to disease immunopathogenesis and severity.

Keywords: Dengue;T lymphocytes;Cellular senescence.

3231 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The Adipokine Resistin Reduces IL-10 Production in Macrophages Infected with *Leishmania braziliensis*

MIRANDA, G.O.; SANTOS, C.P.; DE CARVALHO FILHO, E.M.; DE CARVALHO, L.P.. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL.

Introduction: Cutaneous leishmaniasis (CL) due to *L. braziliensis* is an inflammatory parasitic disease characterized by the development of ulcerated lesions in the skin. High levels of TNF and IL-1 β , cytotoxicity, high parasite load and obesity are associated with poor response to treatment. Adipokines are proteins secreted by adipocytes that may influence inflammatory response. **Objectives:** To determine adipokines levels in obese CL patients and investigate the contribution of adipokines to inflammatory response in *L. braziliensis*-infected macrophages. **Methodology:** Peripheral blood mononuclear cells from healthy subjects (HS), obese and lean CL patients were obtained, and monocyte-derived macrophages were used. **Results:** Obese CL patients produced more leptin and IL-1b, and less adiponectin than lean CL patients in serum. *L. braziliensis*-infected macrophages produced high levels of TNF, IL-1b, IL-6, and moderate IL-10. Addition of the adipokine resistin to *Leishmania*-infected macrophages from HS decreased IL-10 levels. Leptin, adiponectin and resistin did not affect inflammatory response **Conclusions:** Our data show that resistin decreases IL-10 production in *L. braziliensis*-infected macrophages without altering inflammatory response.

Keywords: Cutaneous Leishmaniasis ;Obesity ;Resistin.

3232 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

PULMONARY ALLERGIC-LIKE INFLAMMATORY RESPONSE IS EXACERBATE IN THE ABSENCE OF TLR9 DURING CRYPTOCOCCUS GATTII INFECTION

CARDOSO, M.F.¹; DE ALMEIDA, G.J.M.²; CAVALCANTI, E.J.O.²; LIANI, S.A.C.²; GOMES, A.I.N.²; COVRE, L.P.³; MORROT, A.⁴; PINTO, K.G.⁵; DA SILVA-JUNIOR, E.B.²; FILARDY, A.D.⁵; GUEDES, H.L.D.M.⁵; DECOTE-RICARDO, D.⁶; FREIRE-DE-LIMA, C.G.². 1. IFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. IBCCF/UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. UFES, VITÓRIA - ES - BRASIL; 4. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 5. IMPG/UFRJ, RIO DE JANEIRO - RJ - BRASIL; 6. UFRRJ, RIO DE JANEIRO - RJ - BRASIL.

Cryptococcosis is a potentially systemic mycosis responsible for about 625,000 deaths annually. The species complex *Cryptococcus neoformans*/*C. gattii* are the main etiological agents of the disease, with cryptococcal meningitis being its most severe form. An inflammatory response characterized by eosinophilia, accumulation of Charcot-Leyden crystals and high levels of IL-4 and IL-5 is observed in the lungs of mice infected with *C. neoformans*. However, the pulmonary inflammatory response to *C. gattii* infection is still poorly understood. Recently, our group demonstrated that TLR9 knockout (TLR9^{-/-}) mice are more susceptible to *C. gattii*. TLR9^{-/-}-infected mice exhibit intense fibrosis, accumulation of polymorphonuclear cells and lower levels of IFN- γ and IL-17 in the lung. Our hypothesis is that *C. gattii* lung infection triggers an allergic inflammatory response, which is exacerbated in the absence of TLR9. Thus, we intratracheally inoculated 10⁴ yeast cells of the *C. gattii* virulent strain R265 in C57BL/6 WT and TLR9^{-/-} mice. Our findings reveal increased eosinophilia and monocyte-derived alveolar macrophages (moAMs) in TLR9^{-/-} mice lungs three weeks post-infection.

Histopathology showed accumulation of polymorphonuclear cells and Charcot-Leyden crystals in the lungs of infected TLR9^{-/-} mice. In addition, the TLR9^{-/-} mice showed lower levels of IFN- γ , IL-6 and IL-17 and high levels of IL-4 and IL-5 in the lungs three weeks post-infection by *C. gattii*. These results suggest that pulmonary infection by *C. gattii* induces an allergic-like inflammation and that TLR9 is crucial to maintain the Th1/Th17/Th2 balance. This work contributes to the knowledge of cryptococcosis immunopathology. However, additional studies are needed to elucidate mechanisms involved in cryptococcosis immunopathogenesis and the real role of TLR9 in the course of infection by *C. gattii*.

Keywords: CRYPTOCOCCOSIS;INFLAMMATION;TOLL-LIKE RECEPTOR 9.

3234 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Functional dichotomy of CD4-CD8- T cells with differential expression of CD28 in patients with distinct clinical forms of Chagas disease

NEVES, E.G.A.¹; KOH, C.C.¹; LUCINDA, P.P.D.¹; SILVA, S.D.A.¹; GOLLOB, K.J.²; NUNES, M.D.C.P.¹; DUTRA, W.¹. 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. EINSTEIN HOSPITAL ISRAELITA, SÃO PAULO - SP - BRASIL.

Introduction: DN T cells (CD4⁻CD8⁻) comprise a small population of circulating T lymphocytes and are major sources of pro- and anti-inflammatory cytokines, such as TNF and IL-10, in patients with chronic Chagas cardiomyopathy (CCC) and indeterminate clinical form (IND), respectively. However, the functional characteristics of DN T cells with differential CD28 expressions and their association with Chagas disease pathology remain unknown, highlighting the need for further investigation to understand their potential role in disease progression and outcomes. **Methods:** We evaluated the expression of cytokines, cytokine receptors, cytotoxic molecules, and chemokine receptors in circulating DN TCR $\gamma\delta^{+}$ and $\alpha\beta^{+}$ T cells with differential CD28 expressions from patients with different clinical forms of Chagas disease (CONEP 2.809.859). **Results:** Our findings revealed a higher frequency of DN⁺ CD28⁺ T cells in the peripheral blood of CCC than in IND patients, which exhibited high expression of the CCR5 chemokine receptor and was associated with cytotoxic activity mediated by granzyme A (GzMA). This subset may facilitate migration to inflamed myocardium, as confirmed by *in silico* analyses showing upregulation of CD28, CCR5, and GzMA mRNA in the heart from CCC samples. In contrast, DN⁺ CD28⁻ T cells were more prevalent in the IND group, which was associated with a balanced immune response by a positive correlation between TNF and IL-10 levels and a negative correlation between IL-17A levels and the left ventricular diastolic (LVDD) and systolic diameters (LVSD). **Conclusion:** DN T cells with distinct CD28 profiles play distinct roles in Chagas disease. DN⁺CD28⁺ T cells may drive CCC pathogenesis through inflammatory/cytotoxic activity, while DN⁺CD28⁻ T cells may protect cardiac function by preserving immune balance. The inhibition of the activation of DN T cells may be a strategy to decrease inflammation in Chagas disease cardiomyopathy and improve patient clinical outcomes.

Keywords: Chronic Chagas Cardiomyopathy;Immunopathology;DN T cells .

3239 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Role of human neutrophil extracellular vesicles in endothelial cell activation.

RAMOS, C.D.L.¹; WACLAWIAK, I.¹; WERNECH, M.V.I.¹; COELHO, S.V.A.¹; SILVA, C.A.A.²; COSTA, A.G.B.¹. 1. INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES - IMPG - UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO - UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Neutrophils rapidly infiltrate *Leishmania*-infected tissue *in vivo* and interact with parasites, impacting disease outcome. Studies have shown that neutrophils can release extracellular vesicles (EVs) containing membrane receptors, adhesion molecules, granule proteins, and cytokines. These EVs have multiple functions in neutrophil responses. During inflammation, neutrophil migration from the bloodstream to tissues is orchestrated by interactions with endothelial cells. Our work aims to understand how EVs from *Leishmania*-stimulated neutrophils influence these interactions. We purified EVs from neutrophils, activated or not with *Leishmania amazonensis*, via ultracentrifugation. EVs were characterized by nanoparticle tracking analysis (NTA), transmission microscopy, and western blot. *Leishmania* stimulation increased the release of small EVs (SEVL_a) compared to controls (SEVL_{ct}). Dot blot assays showed positive labeling for CD63 and argonaute protein, with stronger staining in SEVL_a, and negative labeling for cytochrome C in both groups. To assess the effect of EVs on vascular permeability, we carried out a transwell assay using TEER (transepithelial/ transendothelial electrical resistance) and observed that treatment of HBMEC with stimulated SEVL_a resulted in increased endothelial cells permeability compared to both positive control (Badicinin) and negative control. Moreover, SEVL_a pretreatment increased neutrophils adhesion to HBMEC monolayer in comparison to SEVL_{ct} treated neutrophils. Next steps include evaluating adhesion molecule expression on HBMEC and neutrophils, and cytokine and angiogenic factor production (e.g., TNF- α , IL-8, VEGF) by these cells after treatment with EVs. Our results suggest that neutrophil EVs could significantly influence cell migration to inflamed tissues. Additionally, the presence of argonaute protein indicates a potential role for microRNAs in modulating endothelial cell activation.

Keywords: EXTRACELLULAR VESICLES;NEUTROPHIL;ENDOTHELIAL CELL.

3244 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Staphylococcus aureus LukMF' targets neutrophils to promote skin and soft tissue infection

BOFF, D.; NAIK, S.; TORRES, V.J.. NEW YORK UNIVERSITY GROSSMAN SCHOOL OF MEDICINE, NEW YORK - ESTADOS UNIDOS DA AMERICA.

Staphylococcus aureus is a Gram-positive bacterium that colonizes approximately 20 to 30% of the healthy population in the anterior nares and is the most common pathogen isolated from skin and soft tissue infections. The emergence of the methicillin-resistant lineage USA300 accounts for most community-associated SSTI cases in the United States of America, Latin America, and Western Pacific. Owing to its human tropism, mechanisms that enabled the rise of USA300 as a major skin pathogen remain incompletely defined. Unearthing the full spectrum of *S. aureus* virulence factors responsible for SSTIs requires the implementation of new animal models. In nature, *S. aureus* colonizes and infects multiple hosts including rodents. Herein, we used a natural *S. aureus* rodent strain known as DIP to dissect the pathogenesis of SSTIs in a natural host. We show that rodent-adapted *S. aureus* is more virulent in mice compared to human-adapted USA300 in SSTI models. A combination of comparative genomics, proteomics, and in vivo studies uncovered LukMF', a pore-forming leukocidin that is closely related to the human-specific PVL, as the main driver of SSTI pathogenesis. We found that the uncontrolled killing of neutrophils in the skin by LukMF' amplified an inflammatory cascade that fueled bacterial survival with a concomitant exacerbation of pathology. Consistent with these observations, protecting the host from LukMF' by deleting the LukMF' host receptor, temporally depleting neutrophils, and/or vaccinating with LukMF' protected animals from severe SSTI. Together, this work establishes LukMF' as major driver of severe SSTI in rodents. More broadly, this work supports the epidemiological studies that suggest that human-specific leukocidins such as PVL likely contribute to USA300-mediated SSTI pathogenesis.

Keywords: *Staphylococcus aureus*; neutrophils; skin.

3246 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

IMMUNOLOGICAL EVALUATION OF A HYDRAZONE DERIVATIVE (LASSBio-1736) ENCAPSULATED IN ALBUMIN NANOPARTICLES FOR THE EXPERIMENTAL TREATMENT OF VISCERAL LEISHMANIASIS

PEREIRA, P.H.F.¹; DE SOUZA, T.H.F.²; DE ARAÚJO, M.P.²; COLOMBO, F.A.³; NUNES, J.B.³; COELHO, L.F.L.⁴; LIMA, L.M.⁵; MARQUES, M.J.². 1. DEPARTAMENTO DE PARASITOLOGIA E PATOLOGIA (IC) - UNIVERSIDADE FEDERAL DE ALFENAS, ALFENAS - MG - BRASIL; 2. DEPARTAMENTO DE PARASITOLOGIA E PATOLOGIA - UNIVERSIDADE FEDERAL DE ALFENAS, ALFENAS - MG - BRASIL; 3. DEPARTAMENTO DE ANÁLISES CLÍNICAS - UNIVERSIDADE FEDERAL DE ALFENAS, ALFENAS - MG - BRASIL; 4. DEPARTAMENTO DE MICROBIOLOGIA E IMUNOLOGIA - UNIVERSIDADE FEDERAL DE ALFENAS, ALFENAS - MG - BRASIL; 5. INSTITUTO DE CIÊNCIAS BIOMÉDICAS - UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Visceral leishmaniasis (VL) is a potentially fatal neglected tropical disease caused by *Leishmania* protozoa. Current treatments are limited by significant side effects and the emergence of drug resistance, underscoring the urgent need for alternative therapies. This study evaluated the immune response of hamsters infected with *Leishmania (L.) infantum chagasi* and treated with LASSBio-1736—a hydrazone derivative with known leishmanicidal activity—administered either in free form or encapsulated in bovine serum albumin (BSA) nanoparticles. The nanoparticles showed high encapsulation efficiency and suitable physicochemical properties for oral administration. Parasite burden in target organs and cytokine expression (IL-10, IFN- γ , IL-17) in liver and spleen were assessed via qPCR. LASSBio-1736 at 6.25 mg/kg/day did not significantly reduce parasite load. Conversely, Glucantime treatment led to marked parasite burden reduction and increased pro-inflammatory cytokines, including IFN- γ and IL-10. Interestingly, BSA-encapsulated LASSBio-1736 elevated IL-10 and reduced IFN- γ in the liver, suggesting an immunomodulatory effect, which aligned with histological signs of tissue preservation. Notably, empty nanoparticles also induced IL-10, indicating the BSA carrier itself may modulate immune responses. Although LASSBio-1736 did not reduce parasite load at the tested dose, its impact on cytokines and tissue integrity points to immunomodulatory potential. These findings support further studies using higher doses and pharmacokinetic studies to assess absorption. BSA-based nanoparticles appear promising for infectious disease treatment, potentially improving bioavailability and reducing toxicity. This study highlights the value of including immunological parameters in preclinical evaluations to better inform safety and efficacy of experimental VL therapies.

Keywords: visceral leishmaniasis; nanoparticles; cytokines.

3250 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Recombinant *Lactococcus lactis* expressing HSP65 induces regulatory T cells and reduces pathology in murine cutaneous leishmaniasis

GONÇALVES, Í.D.S.¹; ANDRADE, C.M.¹; PILGER, L.M.¹; PINTO, N.D.S.¹; DE SALES, J.L.¹; DA SILVA, G.D.¹; DE OLIVEIRA, E.C.¹; LEAL, A.M.L.D.C.¹; MAIOLI, T.U.²; FARIA, A.M.C.³; BRODSKYN, C.I.¹. 1. LABORATORY OF PARASITE-HOST INTERACTION AND EPIDEMIOLOGY (LAIPHE) - GONÇALO MONIZ INSTITUTE - FIOCRUZ, SALVADOR, BRAZIL, SALVADOR - BA - BRASIL; 2. DEPARTMENT OF NUTRITION, NURSING SCHOOL, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, MINAS GERAIS, BRAZIL, BELO HORIZONTE - MG - BRASIL; 3. DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY, BIOLOGICAL SCIENCES INSTITUTE, FEDERAL UNIVERSITY OF MINAS GERAIS, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, MINAS GERAIS, BRAZIL, BELO HORIZONTE - MG - BRASIL.

INTRODUCTION: Cutaneous leishmaniasis (CL), a neglected tropical disease highly prevalent in Brazil, is caused by *Leishmania braziliensis* and is characterized by ulcerative skin lesions and an exacerbated Th1-mediated inflammatory response. This study investigates the therapeutic potential of oral tolerance (OT) induced by a genetically modified strain of *Lactococcus lactis* (*L. lactis*) producing heat shock protein 65 (HSP65) from *Mycobacterium leprae* in a murine model of CL. **METHODOLOGY:** BALB/c mice were infected with *L. braziliensis* or *L. major*, and received oral treatment with HSP65-expressing *L. lactis* or control *L. lactis* (empty vector) for four consecutive days, starting at four weeks after infection. **RESULTS:** Mice treated with HSP65-expressing *L. lactis* exhibited reduced lesion size and parasite burden. Cytokine analysis in draining lymph nodes revealed a shift from a predominantly pro-inflammatory IFN- γ response towards enhanced IL-10 production, which correlated with reduced inflammation and decreased tissue damage. Additionally, the treatment promoted an increase in regulatory T cells (Tregs), including CD4⁺CD25⁺FOXP3⁺ and CD4⁺LAP⁺ (membrane-associated TGF- β) cells in the draining lymph nodes. This effect was not reproduced in the more severe CL model induced by *L. major* infection. **CONCLUSION:** This study underscores the potential of oral tolerance induction using HSP65-producing *L. lactis* as a promising immunoregulatory therapeutic approach for some chronic inflammatory infections, mainly those ones which immune response display a primed balance.

Keywords: Leishmaniasis; Oral Tolerance; *Lactococcus lactis*.

3255 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Immune Shifts with AW-HRL-C: A Th2-Driven Anti-Schistosomal Path

FIGOIRESE, M.S.¹; GUIMARÃES, V.H.²; ANSELMO, M.C.²; DUARTE, F.O.²; BIANCHI, J.²; DE CORREIA, C.R.S.T.B.³; FRAGELLI, B.D.D.L.⁴; RODOLPHO, J.M.D.A.²; ASSIS, M.⁵; LÍBERO, L.O.⁴; ALLEGRETTI, S.M.⁶; DA SILVA, E.L.⁴; TEIXEIRA, F.R.³; ANIBAL, F.². 1. UNICESUMAR, MARINGÁ - PR - BRASIL; 2. DEPARTMENT OF MORPHOLOGY AND PATHOLOGY, FEDERAL UNIVERSITY OF SÃO CARLOS, SÃO CARLOS - SP - BRASIL; 3. DEPARTMENT OF GENETICS AND EVOLUTION, FEDERAL UNIVERSITY OF SÃO CARLOS, SÃO CARLOS - SP - BRASIL; 4. CENTER FOR DEVELOPMENT OF FUNCTIONAL MATERIALS, FEDERAL UNIVERSITY OF SÃO CARLOS, SÃO CARLOS - SP - BRASIL; 5. DEPARTMENT OF ANALYTICAL AND PHYSICAL CHEMISTRY, UNIVERSITY JAUME I, CASTELLÓ DE LA PLAN - ESPANHA; 6. ANIMAL BIOLOGY DEPARTMENT, STATE UNIVERSITY OF CAMPINAS, CAMPINAS - SP - BRASIL.

Schistosomiasis remains a major public-health challenge in Brazil. In response to the growing threat of parasites loss of sensibility to praziquantel (PZQ), we evaluated in vivo the anti-schistosomal performance of curcumin-anchored silver tungstate (AW-HRL-C), investigating the differences on immune response to the parasite with these compounds. The profile was based on the immunomodulatory role of IgG, IgE and IL-10, main characteristic molecules on schistosomiasis action. To build a more specific recognition of immunoglobulines, adult *Schistosoma mansoni* worms were frozen and then used to obtain a total protein extract by sonication in proteasis inhibition buffer, followed by centrifugation. This extract was used to sensitize plates for sandwich ELISAs assays. The detection of IgG and IgE were made with BD Pharminge Biotin Rat Anti-Mouse IgG2a and IgE, following its instruction guide. To quantify IL-10 levels was used the kit BD Cytometric Bead Array - Mouse Th1/Th2/Th17, following its instruction guide. ELISA assays showed increased IgE levels in the AW-HRL-C group, approximately twice as high as those in positive control (CP), while the PZQ group maintained levels similar to the CP. IgG levels were reduced in all infected groups, with a significant difference observed between the negative (CN) and CP. The AW-HRL-C group showed lower IgG higher than CP. IL-10 levels were slightly reduced in both treated groups, with a more pronounced decrease in the AW-HRL-C group. AW-HRL-C exhibits strong immunomodulatory potential in *S. mansoni* infection, as evidenced by increased IgE levels and reduced IL-10 production. These results suggest activation of a Th2 immune response and point to AW-HRL-C as a promising alternative therapy for schistosomiasis. Its ability to modulate key components of the host immune profile reinforces the need for further studies, aiming to elucidate its action and therapeutic applicability. Process FAPESP: nº 2023/09490-2; 2013/07296-2

Keywords: schistosomiasis; inflammatory diseases; neglected tropical disease.

3264 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Clinical isolates obtained from the Hospital das Clínicas display lower tolerance to capsule-inducing stressors

NETO, J.B.¹; DE SOUZA, R.L.L.²; SANTANA, R.D.C.²; TRINDADE, G.B.²; BASSI, V.¹; DANTAS, B.A.¹; SILVA, J.D.C.²; SILVA, J.S.¹; BOCCA, A.L.³. 1. FIOCRUZ-BI-INSTITUTIONAL TRANSLATIONAL MEDICINE PLATFORM, RIBEIRÃO PRETO - SP - BRASIL; 2. RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. UNIVERSITY OF BRASÍLIA, BRASÍLIA - DF - BRASIL.

Cryptococcus neoformans and *Cryptococcus gattii* are encapsulated fungi that can cause severe opportunistic infections, including cryptococcal meningitis. Although extensively studied in laboratory models, little is known about the phenotypic profiles of clinical isolates circulating in specific regions of Brazil. In this study, we aim to characterize the virulence factors of *C. neoformans* and *C. gattii* using clinical isolates provided by the Hospital das Clínicas of FMRP-USP, with the goal of establishing a representative pathogenic profile of the strains circulating in the Ribeirão Preto region. To achieve this, we performed phenotypic assays targeting capsule production in inductive culture media such as Littmann and Low Iron Media, melanin synthesis in Niger seed and epinephrine media, as well as resistance tests to osmotic, cell wall, and oxidative stress conditions. So far, 17 clinical isolates have been evaluated, revealing significant phenotypic diversity. We observed variations in melanin production and capsule size, suggesting potential heterogeneity in the expression of virulence-associated genes. Preliminary stress test results indicate that, compared to reference wild-type strains, the clinical isolates generally exhibit lower resistance to the applied stress conditions. These findings suggest that the persistence of the fungi in the host environment may not impose intense selective pressure, potentially allowing genetic drift to contribute to the emergence of less adapted fungal profiles. The experiments are ongoing, with additional analyses planned to deepen the understanding of these differences and their possible clinical and epidemiological implications. Keywords: *Cryptococcus*; Capsule production; Virulence factor.

3286 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Extracellular vesicles therapy released by virulent *Leishmania amazonensis* improves immune response against experimental parasite infection

DE OLIVEIRA, S.R.; VIDAL, A.S.; ZAULI, R.C.; DUENHA, E.D.S.; TORRECILHAS, A.C.T.; XANDER, P. UNIFESP, DIADEMA - SP - BRASIL.

The treatment for leishmaniasis is still a challenge since drugs have high toxicity and adverse effects, impacting treatment adherence. Thus, the development of new therapeutic alternatives can improve the quality of life and treatment of patients. Recent studies showed that extracellular vesicles (EVs) released by *Leishmania amazonensis* can impact the response of macrophages, B-1 cells, and neutrophils, and can protect mice in immunization protocols. However, the therapeutic potential of these EVs in cutaneous leishmaniasis has not yet been evaluated. This study aimed to assess the effect of *L. amazonensis* EVs released by parasites with different virulence profiles in the treatment of experimental leishmaniasis. BALB/c mice were infected with *L. amazonensis* with a virulent profile (in vivo derived parasites - IVD-P). After the initial lesion appeared, the animals were treated with EVs produced by *L. amazonensis* with an attenuated profile (LT-P EVs) or with EVs from parasites with a virulent profile (IVD-P EVs). Animals were intraperitoneally treated with 4 µg of EVs/animal for 4 weeks according to the following therapeutic scheme: two doses at 15-day intervals or three doses at 7-day intervals. Lesion sizes were evaluated weekly and showed a significant reduction in the group treated with 3 doses of IVD-P EVs, compared to the control group and the group treated with LT-P EVs. The parasite load showed a significant reduction in animals treated with IVD-P EVs in both treatment protocols (2 doses and 3 doses). In addition, a reduction in the inflammatory milieu in lesions was observed in animals treated with IVD-P EVs. Animals treated with LTP-EVs or IVDP-EVs had higher levels of IgG1 to total *L. amazonensis* antigens. In contrast, the highest levels of IgG2a were detected in animals treated with IVDP-EVs. In conclusion, our results showed a new potential therapeutic approach based on *L. amazonensis* EVs for cutaneous leishmaniasis.

Keywords: Extracellular vesicles; *Leishmania amazonensis*; therapy.

3291 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Immunodiagnostic Trends and Regional Variability in the Management of Visceral Leishmaniasis in Brazil (2007–2023): Insights from National Surveillance Data

DE SANTANA, M.B.R.¹; TEIXEIRA, H.M.P.¹; SOUZA, J.L.F.M.¹; FRAGA, D.B.M.¹; BRODSKYN, C.I.¹; DE MENEZES, J.P.B.¹; VERAS, P.S.T.²; DAVID, R.B.²; OLIVEIRA, V.D.A.²; VERAS, P.S.T.³. 1. LABORATORY OF HOST-PARASITE INTERACTION AND EPIDEMIOLOGY (LAIPHE) – GONÇALO MONIZ INSTITUTE – OSWALDO CRUZ FOUNDATION (FIOCRUZ), SALVADOR - BA - BRASIL; 2. CENTER FOR INTEGRATION OF DATA AND KNOWLEDGE FOR HEALTH - OSWALDO CRUZ FOUNDATION (FIOCRUZ), SALVADOR - BA - BRASIL; 3. LABORATORY OF HOST-PARASITE INTERACTION AND EPIDEMIOLOGY (LAIPHE) – GONÇALO MONIZ INSTITUTE – OSWALDO CRUZ FOUNDATION (FIOCRUZ); NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY FOR TROPICAL DISEASES (INC, SALVADOR - BA - BRASIL.

Background: Visceral leishmaniasis (VL) remains a major public health challenge in Brazil, with persistent endemicity and significant regional disparities. Immunological diagnosis plays a critical role in the early identification and management of VL, particularly in areas where parasitological methods are limited. This study aimed to assess temporal trends in the use of immunodiagnostic methods and to map treatment patterns across Brazil using national surveillance data.

Methods: Data of 55,573 individuals with VL were extracted from the Notifiable Diseases Information System (SINAN) for the period 2007–2023. Analyses were performed using R software, focusing on proportions of case entry types, diagnostic modalities (parasitological, immunofluorescence assay [IFI], and other immunological methods), and first-line treatments. Regional trends and shifts in clinical management were also explored.

Results: From 2007 to 2023, VL diagnoses using other immunological methods rose from 12% to 49%, while IFI use declined from 49% to 29%, indicating a shift in diagnostic approaches. Many cases, especially early in the period, lacked diagnostic confirmation. Treatment patterns also changed: pentavalent antimonials were predominant until 2015, but liposomal amphotericin B became the main first-line therapy after 2020, reaching over 50% by 2023. Interestingly, relapse case entries increased from 2% to 11%. Regional differences in diagnostic and treatment practices likely reflect disparities in access to laboratory and therapeutic resources.

Conclusion: The increasing use of immunological diagnostics and liposomal amphotericin B reflects progress in VL management in Brazil. However, persistent gaps in diagnostic access and a rise in relapse cases highlight the need to strengthen immunodiagnostic capacity and ensure equitable care, especially in underserved regions.

Keywords: visceral leishmaniasis; immunodiagnosis; SINAN.

3296 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Capsule-Mediated Modulation of Host Immune Responses and Tissue Damage during *Legionella longbeachae* Infection

CÂMARA, P.H.M.¹; MASCARENHAS, D.P.A.¹; BUCHRIESER, C.²; ZAMBONI, D.S.¹. 1. UNIVERSIDADE DE SÃO PAULO - USP, RIBEIRÃO PRETO - SP - BRASIL; 2. INSTITUT PASTEUR, PARIS - FRANÇA.

Bacteria of the genus *Legionella* are intracellular, Gram-negative organisms that cause a severe form of pneumonia known as Legionnaires' disease. Among the most notable species, *Legionella longbeachae* stands out as an important agent in outbreaks worldwide, despite being relatively understudied. Studies suggest that *L. longbeachae* displays greater lethality and replication in mice lung than other *Legionella* species; however, the factors underlying these observations remain unclear. Genomic and transcriptomic analyses have revealed that this species possesses distinct virulence factors compared to other *Legionella spp.*, notably the presence of a capsule, an unprecedented virulence factor within the *Legionella* genus, with limited exploration in the pathogenesis of Legionnaires' disease. Based on these findings, this study aims to investigate the role of the capsule in the pathogenicity of *L. longbeachae* by comparing the wild-type bacteria to a mutant deficient in capsule biosynthesis genes. The results indicate that, in early stages of infection, the capsule enhances pro-inflammatory cytokine production and promotes cellular recruitment, especially of neutrophils. In subsequent stages of infection, the capsule promotes greater activation of neutrophil extracellular traps (NETs) and exacerbated pulmonary and hepatic tissue damage, despite only minor differences in bacterial replication within the lungs or dissemination to the liver. The increase in tissue damage also precedes the lethality of the bacteria which is entirely dependent on the presence of the capsule. Altogether, these findings highlight the capsule as a key factor in *L. longbeachae* infection. Future studies will aim to elucidate the mechanisms underlying the increased tissue damage observed in the lungs of infected animals, with focus on the hypothesis that the capsule may directly induce cell damage or promote it via NET-dependent mechanisms.

Keywords: *Legionella longbeachae*; Capsule; Host-pathogen interaction.

3298 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Unraveling metabolic reprogramming in human monocytes during *Leishmania amazonensis* and *Leishmania braziliensis* infection: paving the way for novel therapies.

LEAL, A.M.L.D.C.¹; DA SILVA, G.D.¹; DE OLIVEIRA, E.C.¹; DE SALES, J.L.¹; ANDRADE, C.M.¹; GONÇALVES, Í.D.S.¹; DE SOUZA, N.¹; PILGER, L.M.¹; VIEIRA, P.M.M.D.M.²; BRODSKYN, C.I.¹. 1. FIOCRUZ BAHIA - INSTITUTO GONÇALO MONIZ (IGM), SALVADOR - BA - BRASIL; 2. UNICAMP, CAMPINAS - SP - BRASIL.

One of the features of intracellular survival of *Leishmania* parasites relies on its ability to reprogram host cell metabolism. Emerging evidence suggests that metabolic adaptation is essential for sustaining infection and could identify new targets for therapeutic candidates. In this study, we investigate how *L. amazonensis* and *L. braziliensis* modulate the metabolism of human monocytes, key effector cells in early infection.

Monocytes, isolated from the peripheral blood of healthy donors, were infected with *Leishmania* spp. for 18 hours. We used Seahorse to measure Extracellular Acidification Rate (ECAR) and Oxygen Consumption Rate (OCR). To evaluate the role of specific metabolic pathways in supporting infection, cells were pre-treated with inhibitors of glycolysis, mitochondrial and lipid metabolism prior to infection, and parasite load was quantified. Mitochondrial mass and membrane potential were evaluated using MitoTracker probes, and reactive oxygen species (ROS) production was monitored with MitoSOX and CellROX.

Our findings reveal that *L. amazonensis* and *L. braziliensis*-infected monocytes exhibit similar metabolic profiles, marked by reduced ECAR and OCR rates compared to uninfected controls. Treatment with metabolic inhibitors significantly reduced parasite load while enhancing cellular and mitochondrial ROS production. Given that ROS is a key leishmanicidal mechanism, this suggests that metabolic inhibition may impair parasite survival not only by disrupting nutrient availability but also by potentiating host microbicidal responses. Furthermore, increased mitochondrial mass and membrane potential suggest induction of mitochondrial biogenesis during infection.

These results indicate that although the metabolic alterations induced by both species are comparable, they play a critical role in sustaining intracellular infection. Targeting host cell metabolism thus emerges as a promising avenue for host-directed therapeutic development against leishmaniasis.

Keywords: Immunometabolism; *Leishmania*; Monocyte.

3303 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Biological Sex Modulates Systemic and Mucosal-Associated Immune Responses During Acute SARS-CoV-2 Infection

DE JESUS, T.A.; NETO, P.V.D.S.; LUCAS, J.M.; NETO, F.M.D.S.; JÚNIOR, R.B.M.; CARDOSO, C.R.D.B.. UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Introduction: Long-term manifestations of SARS-CoV-2 infection affect millions of COVID-19 survivors. The immune response to SARS-CoV-2 infection exhibits significant sex-based differences, influencing both the acute phase of the disease and its long-term consequences. Among the persistent sequelae, gastrointestinal symptoms are frequently reported. SARS-CoV-2 can infect intestinal cells, disrupt gut microbiota homeostasis, and induce sustained mucosal inflammation. Similar factors are implicated in the development and recurrence of Inflammatory Bowel Diseases (IBD), such as Crohn's disease and ulcerative colitis, highlighting pathophysiological overlaps between viral infection and intestinal disorders. **Objective:** This study aimed to investigate the relationship between SARS-CoV-2 infection and the activation of intestinal inflammatory processes, with a focus on the influence of biological sex on systemic and local immune responses. **Methods:** To this end, male and female K18-hACE2 mice were assessed during the acute phase of infection (4 days post-inoculation). **Results:** Infection inhibited weight gain in females, while also induced peripheral lymphopenia in both sexes, accompanied by elevated neutrophil counts in females and increased monocytes in males. No significant differences were detected in IL-6, IL-1 β , TNF- α , or IFN- γ levels in the lungs or colon of infected animals. However, infected males exhibited lower lung IL-17 levels compared to females, suggesting a potential sex-specific regulation of local inflammatory pathways. **Conclusion:** These findings indicate that the immune response to acute SARS-CoV-2 infection involves subtle yet distinct systemic and tissue-specific differences between sexes, consistent with the opposing effects of sex hormones on leukocyte dynamics that may influence long-term outcomes.

Financial Support: FAPESP, CAPES, CNPq

Keywords: SARS-CoV-2; Mucosal immunity; Sex differences.

3311 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

EVALUATION OF SIGNALING PATHWAYS INVOLVED IN CARDIAC FIBROSIS INDUCED BY T. CRUZI INFECTION HIGHLIGHTED DYRK2 AS A NOVEL CHEMOTHERAPEUTIC TARGET FOR CARDIOMYOPATHY

CONTINENTINO, A.L.; XAVIER, Í.D.L.D.S.; MOREIRA, G.L.D.S.; DE SOUZA, P.H.O.; NETO, D.T.D.S.; NOGUEIRA, A.D.R.; DE MESQUITA, L.B.; DE SOUZA, R.S.D.; ALVAREZ, C.M.C.. CELLULAR ULTRASTRUCTURE LABORATORY - OSWALDO CRUZ INSTITUTE - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Chagas disease is a neglected tropical disease and the leading cause of non-ischemic cardiomyopathy in Latin America. *Trypanosoma cruzi* induces extracellular matrix deposition in cardiac tissue, leading to fibrosis associated with reduced ventricular ejection and arrhythmias. Thus, combination therapies with trypanocidal and antifibrotic agents are needed. Previous studies from our group highlighted DYRK2, AMPK2, TGF- β , and p38 pathways during chronic infection in vivo. To validate these as fibrosis targets, we evaluated LDN193189 (TGF- β /p38 inhibitor), LDN192960 (DYRK2 inhibitor), and GSK621 (AMPK activator) during in vitro *T. cruzi* infection. Cytotoxicity (CC50) and trypanocidal activity (IC50) were determined by quantifying VERO cells and intracellular amastigotes (Dm28cluc strain, expressing luciferase). LDN193189 showed CC50 = 8.5 μ M, IC50 = 1.79 μ M; LDN192960, CC50 = 19 μ M, IC50 = 3.02 μ M; GSK621, CC50 = 60 μ M, IC50 = 7.05 μ M. For antifibrotic analysis, mixed culture of cardiomyocytes (MC) and purified cardiac fibroblasts (CF) from fetal mouse hearts were used as 2D monolayers and 3D spheroids, infected with Y strain. Fibrosis was assessed by collagen quantification (Sirius Red/Fast Green assay) and fibronectin deposition visualization (immunofluorescence). In 2D CFs, collagen inhibition showed IC50 of 0.1 μ M (LDN193189), 2.1 μ M (LDN192960), and 1.6 μ M (GSK621). LDN193189 had the highest selectivity index (SI = 85), followed by GSK621 (SI = 63) and LDN192960 (SI = 28). In 3D CMs, LDN193189 and LDN192960 were effective without cytotoxicity; and GSK621 had no effect. In 3D CFs, all compounds reduced collagen and fibronectin. Finally, Western blot of infected 2D CMs showed increased DYRK2 at 24h and 48h, supporting its role and the effect of the inhibitor LDN192960. We conclude that DYRK2 has potential as a new target for treating chronic cardiomyopathy in Chagas disease.

Keywords: Chagas disease; T; cruzi; cardiac fibrosis.

3312 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Analysis of Genetic Polymorphisms in IL10 in Symptomatic and Asymptomatic Chikungunya Virus Infections in a Brazilian Population

COSTA, E.I.F.S.¹; DE LIMA, L.C.¹; DE ANDRADE, C.M.¹; SILVA, H.D.S.¹; SILVA, K.L.M.¹; TEIXEIRA, M.D.G.L.C.¹; SKALINSKI, L.M.²; SANTIAGO, M.S.I.D.C.¹; COSTA, R.D.S.¹; CARLETTO, T.D.O.T.M.¹; FIGUEIREDO, C.¹; CARNEIRO, V.L.³. 1. UFBA, SALVADOR - BA - BRASIL; 2. UESC, SALVADOR - BA - BRASIL; 3. UNEB, SALVADOR - BA - BRASIL.

Chikungunya fever is an arboviral disease caused by the Chikungunya virus (CHIKV), transmitted primarily by *Aedes aegypti* and *Aedes albopictus*. Infection may lead to high fever, severe joint pain, and in some chronic cases, persistent arthritis. The presence of anti-CHIKV IgG antibodies indicates prior exposure and potential immunity. Genetic predisposition may also influence the immune response to infection. This study aimed to investigate the association between *IL10* gene polymorphisms and the presence or absence of persistent symptoms following CHIKV infection in a Brazilian population. We conducted a cross-sectional study involving 612 individuals from Feira de Santana, Bahia. IgG levels were measured by ELISA, and genotyping was performed in 200 seropositive (IgG+) and 412 seronegative (IgG-) individuals. Genetic association analyses were conducted for the *IL10* rs1800871 polymorphism using logistic regression models in PLINK v1.9. A significant association with CHIKV seropositivity was observed under the dominant model (OR = 1.59; 95% CI: 1.10–2.30; p = 0.01) and the additive model (OR = 1.32; 95% CI: 1.01–1.73; p = 0.04). Non-parametric analyses (SPSS and GraphPad) revealed that individuals with the GG genotype had significantly higher plasma IL-10 levels compared to A allele carriers (AG + AA) (p = 0.017). Furthermore, individuals without arthralgia exhibited increased IL-10 production. These findings are supported by data from the GTEx portal, which show that the A allele is associated with reduced *IL10* expression in whole blood, reinforcing its potential role as a negative regulator of anti-inflammatory responses. Our results suggest that genetic variation in *IL10* may influence the immunological profile and clinical outcome of CHIKV infection. Functional studies are warranted to elucidate the underlying mechanisms involved in disease pathogenesis.

Keywords: CHIKV; IL-10 PLASMA LEVELS; POLYMORPHISMS.

3316 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

ASSESSMENT OF SUSCEPTIBILITY TO DENGUE WITH WARNING SIGNS IN OFFSPRING BORN OF Zika virus-INFECTED MICE

DINIZ, L.N.A.; AZEVEDO, L.M.S.; OLIVEIRA, S.N.; DIAS, M.V.; SILVA, M.E.F.; AMARAL, B.S.; RIBEIRO, V.G.; QUEIROZ JUNIOR, C.; COSTA, V.V.; TEIXEIRA, M.M.; DE SOUZA, D.D.G.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

The Zika virus (ZIKV) caused an epidemic in Brazil in 2015, during which an increase in cases of microcephaly in newborns was observed. Furthermore, a series of fetal malformations has been associated with infection by this virus, a condition known as Congenital Zika Syndrome. More recently, a significant increase in severe dengue cases has been reported. Infection with dengue virus (DENV) can result in symptoms ranging from self-limiting illness to clinical presentations of dengue with warning signs (DWS), which include alterations in hemodynamic parameters. In this context, DWS cases may be linked to prior infections, such as ZIKV, particularly considering the cocirculation of DENV and ZIKV and embryonic exposure to ZIKV. Therefore, the objective of this study was to investigate the association between embryonic ZIKV infection and susceptibility to DWS in adulthood. For this purpose, pregnant C57BL/6 female mice were infected with ZIKV or injected with PBS via intraperitoneal route on embryonic day 5.5. Six weeks after the natural birth of the offspring, the animals were injected intravenously with DENV or PBS and euthanized after 48 hours. The groups were obtained: NI (not infected); NI/DENV; ZIKV/NI; ZIKV/DENV. Blood, plasma, spleen, and liver samples were collected for analysis. Platelet, red blood cell, hemoglobin counts, and hematocrit levels were reduced in the ZIKV/DENV group compared to the other groups, indicating possible hematological alterations. Additionally, macrophage recruitment in the liver and CCL5 levels in the spleen were increased in the ZIKV/DENV group, while splenic vascular permeability was lower when both infections were present. An increase in inflammatory infiltrates was also observed in the liver of DENV-infected animals. Thus, these findings indicate that congenital ZIKV infection may exacerbate the pathophysiological response to DENV infection in adulthood, potentially contributing to greater disease severity.

Keywords: Dengue virus; Zika virus; Dengue with warning signs.

3318 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Obesity and female sex associate with increased post-COVID-19 symptom burden.

RIBEIRO, B.V.¹; BASTOS, R.P.D.S.¹; MARINHO, A.I.¹; ARAUJO, S.N.D.O.¹; SILVA, T.C.²; BISPO, R.³; KHOURI, R.¹; BRODSKYN, C.I.¹; BOAVENTURA, V.¹; DA SILVA, I.B.S.¹; TAVARES, N.M.¹. 1. FIOCRUZ, SALVADOR - BA - BRASIL; 2. LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, LONDON - INGLATERRA; 3. UFBA, SALVADOR - BA - BRASIL.

Post-COVID-19 syndrome (PCS), defined as persistent symptoms lasting beyond 12 weeks after acute SARS-CoV-2 infection, represents a significant public health challenge. While metabolic comorbidities are well-established risk factors for severe acute COVID-19, their impact in PCS remain poorly understood. We conducted a prospective cohort study of 554 COVID-19 survivors (232 obese and 322 non-obese controls) followed for 3 months at a Brazilian tertiary center. Obese patients demonstrated increased symptom persistence at the 2-month follow-up, reporting a median of 4 symptoms (IQR 2–6) versus 3 (IQR 2–5) in non-obese controls ($p = 0.0248$). Nearly 45% of obese participants reported 5 or more concurrent symptoms compared to 26.6% of controls ($p = 0.0002$). Systemic inflammation, as measured by C-reactive protein (CRP), remained elevated in obese individuals (mean 8.0mg/L [SD 5.9] vs. 5.0mg/L [SD 1.9]; $p = 0.0001$), despite their values are below 10mg/L (the threshold for inflammation). Sex-stratified analysis revealed that obese women reported higher symptom counts (median 5 [IQR 3–6] vs. 4 [IQR 2–5]; $p = 0.0186$) and low-grade, but persistent systemic inflammation (mean 8.8mg/L mg/L [IQR 6.0] vs. 5.2mg/L [SD 0.8]; $p = 0.0003$) compared to non-obese women. These findings suggest that obesity may serve as an independent predictor of PCS outcomes, with women being disproportionately affected. The persistent low-grade inflammatory state observed suggest potential mechanisms for targeted interventions in post-COVID-19 care.

Keywords: COVID-19; Long COVID; Obesity.

3321 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Antiviral Immune Mechanisms Underlying the Protective Effects of a Bacterial Lysate in Respiratory Syncytial Virus Infection

BORGES, S.G.¹; ANTUNES, K.H.¹; POPPE, J.G.¹; DA SILVA, R.P.¹; CASTRO, M.S.¹; STEIN, R.T.¹; PASQUALI, C.²; DE SOUZA, A.P.D.¹. 1. PUCRS, PORTO ALEGRE - RS - BRASIL; 2. OM PHARMA, MEYRIN - SUICA.

Introduction: Bronchiolitis is an inflammatory airway disease primarily caused by respiratory syncytial virus (RSV), a pathogen responsible for approximately 190,000 deaths annually among children worldwide. Although vaccines are now available and contribute to reducing mortality, there are still no immunizations for children over six months of age, which exacerbates the public health burden. Broncho-VaxomTM (OM-85) is a bacterial lysate derived from 21 respiratory pathogenic strains and has demonstrated efficacy against bacterial and viral respiratory infections. It induces type I interferon (IFN-I)-mediated antiviral responses and anti-inflammatory effects through various immunological mechanisms, including modulation of dendritic cells, T cells, and macrophages. Previous studies have shown that preventive intranasal treatment with OM-85 is effective against RSV infection. In this project, we aim to evaluate the protective effects of nasal OM-85 administration, an approach with greater translational potential, and to investigate the role of the antiviral immune response.

Methods: Specific pathogen-free (SPF) male BALB/c mice were treated with 2 daily nasal doses of OM-85 (0.5 mg) 5 days before infection. Mice were intranasally infected with RSV (10⁶ PFU/mL) and received a booster dose of OM-85. Additional experiments were conducted using the same protocol, but with intranasal administration of anti-IFNAR 12 hours prior to infection. Analyses were performed 4 days post-infection. The study was approved by the PUCRS Animal Ethics Committee.

Results and Conclusions: Preventive airway administration of OM-85 significantly reduced RSV-induced disease in mice, reducing weight loss and inhibiting viral replication. OM-85 treatment also enhanced the expansion of alveolar macrophages and dendritic cells, even in the absence of IFNAR signaling during the infection. These findings suggest that Broncho-VaxomTM represents a promising prophylactic approach for RSV infection.

Keywords: Respiratory virus;OM-85;Type I interferon.

3342 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Evaluation of the action of leptin and vitamin D on the production of NO by murine macrophages

JANUZZI, L.L.R.¹; TOMÉ, F.D.²; GAMEIRO, J.¹; SCOPEL, K.K.G.¹; NAGIB, P.R.A.¹. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE GOIÁS, JUIZ DE FORA - MG - BRASIL.

Macrophages are key cells in the innate immune response and play a central role in both the defense and pathogenesis of malaria. Previous data from our group demonstrated that in the murine model with *Plasmodium berghei* NK65, there is a compensatory increase in leptin associated with an increase in pro-inflammatory cytokines. Leptin plays a critical role as an immune modulator, primarily related to the pro-inflammatory function of immune cells, including macrophages. Vitamin D3 is also described as a factor active in macrophage activation and correlated with leptin production pathways. Therefore, this study evaluated, in vitro, the action of macrophage polarization (RAW264.7) in the elimination of red blood cells infected with the NK65 strain and the effect of treating these macrophages with leptin and vitamin D3. *P. berghei* NK65 showed resistance to nitric oxide (NO) production, and the M2 profile was not sustained during infection, with increased iNOS expression and high NO production. Leptin treatment resulted in increased NO production in M1 and M2 macrophages. However, NO production was even more evident with vitamin D treatment. Interestingly, the combined treatment appears to have a synergistic effect on NO production. Intracellular iNOS staining also accompanied the NO data. It is expected that the increased NO production may act to eliminate or decrease *Pb* NK65 resistance mechanisms. Our data demonstrate a previously undescribed synergistic effect between the leptin and vitamin D pathways on NO production. Further investigation into these pathways will be carried out.

Keywords: leptin;Macrophage;malaria.

3344 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

METABOLIC AND IMMUNOMODULATORY EFFECTS OF ATOVAQUONE IN TOXOPLASMA GONDII-INFECTED THP-1 CELLS

DE JESUS, M.D.S.¹; NARDINI, V.²; PEREIRA, L.M.¹; VERONEZES, R.M.¹; DE CARVALHO, M.F.¹; VERRI, M.P.¹; YATSUDA, A.P.¹; FACCIOLI, L.H.¹. 1. FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL; 2. DEPTO. DE QUÍMICA DA FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL.

Some parasitic infections are considered neglected diseases and pose a serious global public health concern. *Toxoplasma gondii*, a protozoan from the phylum Apicomplexa, is the causative agent of toxoplasmosis, which has a high prevalence in Brazil (65% to 91%). In immunocompetent individuals, the infection is usually asymptomatic, but it can be severe in newborns and immunocompromised individuals, affecting the lungs, brain, muscles, and eyes. Transmission can occur congenitally, through ingestion of contaminated food or water, or via blood transfusion and organ transplantation. The parasite's life cycle includes three infective forms: tachyzoites (acute phase), bradyzoites (chronic phase), and sporozoites (produced in felines, the definitive hosts). The immune response against *T. gondii* involves both innate and adaptive components. Immune cells are initially activated and trigger the release of pro-inflammatory cytokines, such as IL-12 and IFN- γ , which are essential for pathogen control. However, the parasite can modulate this response by targeting suppressor proteins (SOCS1, SOCS3) and transcription factors like STAT3. Atovaquone, a commonly used drug, blocks the parasite's mitochondrial electron transport chain. This study aims to explore not only atovaquone's known antiparasitic activity but also its potential direct immunomodulatory effects on host cells, through cytokine release analysis. In vitro cytotoxicity and proliferation assays were conducted using PMA-stimulated THP-1 cells, along with cytokine quantification by ELISA. Preliminary results showed low cytotoxicity and significant inhibition of parasite proliferation from 0.125 nM, an important finding for defining the working dose. ELISA assays for IL-1 β also demonstrated a marked reduction in its production, suggesting a potential immunomodulatory effect of atovaquone.

Keywords: *Toxoplasma gondii*; Atovaquone; immunomodulatory.

3356 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Association of Extracellular Vesicles with Uncomplicated Malaria in Individuals Living in Endemic Areas of the Brazilian Amazon

PONTE, M.S.G.; DE CASTRO, K.K.G.; DE LUCA, P.M.; TOTINO, P.R.R.; DANIEL-RIBEIRO, C.T.; RODRIGUES-DASILVA, R.N.; BAPTISTA, B.D.O.; LIMA-JUNIOR, J.D.C.; BERTHO, A.L.. INSTITUTO OSWALDO CRUZ (IOC/FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

Malaria remains a significant public health challenge in endemic regions such as the Brazilian Amazon. Predominantly caused by *Plasmodium vivax* and *Plasmodium falciparum*, the infection triggers immune and cellular responses increasingly associated with extracellular vesicles (EVs). These EVs have emerged as potential biomarkers due to their roles in immune modulation and intercellular communication. This study aimed to quantify plasma levels of EVs derived from erythrocytes and platelets, and to investigate their association with infection dynamics in individuals living in malaria-endemic areas of Brazil (n = 53). Additionally, we evaluated their correlation with circulating levels of IL-6, IL-10, and TNF- α . Plasma samples from individuals infected with *P. vivax* or *P. falciparum*, as well as from healthy endemic and non-endemic controls, were analyzed. EVs were quantified and phenotyped by nano-flow cytometry (CytoFLEX – Beckman Coulter), and cytokine concentrations were measured using Cytometric Bead Array (BD Biosciences). Assessing EV concentrations and phenotypes, we observed higher frequencies of erythrocyte-derived EVs (CD235a⁺) in *P. vivax* (median = 12.56%, IQR: 9.430–17.89) and *P. falciparum* (10.28%, IQR: 8.588–16.89) patients compared to endemic (4.84%, IQR: 2.725–6.010) and non-endemic (3.44%, IQR: 2.520–4.003) controls (p < 0.0001 for both comparisons). Platelet-derived EVs showed no significant differences between groups. Positive correlations were observed between CD235a⁺ EVs and IL-10 levels in *P. vivax* infections (r = 0.40), with similar trends noted for IL-6 and TNF- α in *P. falciparum* infections. These findings suggest that erythrocyte-derived EVs may serve as potential players in the pathophysiological processes underlying uncomplicated malaria in endemic populations. Their association with infection dynamics and circulating cytokine levels highlights their relevance as accessible indicators of host–parasite interactions and immune modulation.

Keywords: *Plasmodium*; Extracellular Vesicles; Flow Cytometry.

3362 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Association between CD4⁺ T-lymphocyte count and SARS-CoV-2 inhibitory response in older adults living with HIV

TOQUETÃO, M.V.L.¹; RIBEIRO, L.B.¹; COELHO, M.P.¹; THOMAZELLA, M.V.¹; KALLÁS, E.G.²; ANTONIO, M.B.¹; NOGUEIRA, R.S.³; MADRUGA, J.V.R.³; BRITES, C.⁴; LUZ, E.⁴; SHEPHERD, B.⁵; LANDAY, A.⁶; CASTILHO, J.L.⁷; VELOSO, V.G.⁸; CARDOSO, S.W.⁸; GRINSZTEJN, B.⁸; SILVEIRA, C.G.T.¹. 1. DEPARTMENT OF INFECTIOUS AND PARASITIC DISEASES, FACULDADE DE MEDICINA, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL; 3. CRT-DST/AIDS-SP (STD/AIDS-SP REFERENCE AND TRAINING CENTER), SÃO PAULO - SP - BRASIL; 4. COMPLEXO HOSPITALAR UNIVERSITÁRIO PROFESSOR EDGARD SANTOS (HUPES), SALVADOR - BA - BRASIL; 5. DEPARTMENT OF BIOSTATISTICS, VANDERBILT UNIVERSITY MEDICAL CENTER, NASHVILLE - ESTADOS UNIDOS DA AMERICA; 6. DEPARTMENT OF MEDICINE, UNIVERSITY OF TEXAS MEDICAL BRANCH GALVESTON, GALVESTON - ESTADOS UNIDOS DA AMERICA; 7. DIVISION OF INFECTIOUS DISEASES, DEPARTMENT OF MEDICINE, VANDERBILT UNIVERSITY MEDICAL CENTER, NASHVILLE - ESTADOS UNIDOS DA AMERICA; 8. INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS, FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

The increased life expectancy of people living with HIV (PLHIV), a result of successful antiretroviral therapy, highlights the importance of understanding their vaccine-induced immune response, a need amplified by the COVID-19 pandemic. This study evaluated the association between CD4⁺ T-lymphocyte counts and the neutralizing antibody (nAb) response against SARS-CoV-2 in an aging cohort of PLHIV. We analyzed plasma from 702 participants aged ≥50 years from the Longitudinal Study of HIV & Aging in Brazil (ELEA-Brazil), collected from 2022 to 2023. nAb levels against Wuhan and variant strains of SARS-CoV-2 were quantified via an electrochemiluminescence-based pseudoneutralization assay. Data were retrieved from the ELEA-Brazil cohort database. A multivariable generalized additive model was used to identify predictors of the nAb response. The model included mean CD4⁺ count, CD4⁺ nadir, age, smoking status, and a smoothed interaction term for vaccination capturing the combined effect of time since last dose across the number of doses. These variables explained 31.8% of the deviance in nAb inhibition. Higher mean CD4⁺ counts ($p=0.0047$) and older age ($p=0.0002$) were positively associated with stronger nAb responses. Conversely, smoking was linked to a lower response ($p=0.0005$). The CD4⁺ nadir count showed a non-linear effect, with a negative correlation at lower levels that reached a plateau at higher counts ($p=0.0091$). Vaccination timing and number of doses were strongly associated with nAb responses across all groups ($p<0.001$). In conclusion, these data indicate that both current (mean) and historical (nadir) CD4⁺ counts are key predictors of the humoral response to SARS-CoV-2 in older PLHIV, presenting distinct linear and non-linear effects. Age, smoking, and vaccination regimen also play crucial roles in shaping this response. These findings emphasize the necessity for tailored vaccination strategies and smoking cessation efforts targeting older PLHIV.

Keywords: PLHIV; Aging; Neutralizing Antibody Response.

3363 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Characterization of the immune response during *Leishmania infantum* infection in mice.

RIBEIRO, H.C.G.; DE SÁ, B.D.S.; PORCINO, G.N.; GUEDES, H.L.D.M.; MARTINS, A.M.D.F.. INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES - UFRJ / INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis comprises a group of diseases caused by parasites of the genus *Leishmania*, which infect host phagocytic cells and become obligate intracellular pathogens. The disease is endemic in approximately 98 countries, with at least 20 pathogenic species identified in humans, presenting in various clinical forms, including localized cutaneous, diffuse cutaneous, mucocutaneous, and visceral leishmaniasis. Previous studies from our group have shown that *Leishmania amazonensis* can induce an immunosuppressive environment during cutaneous infection, leading to T cell exhaustion characterized by impaired effector function, reduced proliferative capacity, and upregulation of inhibitory immune checkpoint receptors. In this study, we investigated the immune response during visceral infection by *Leishmania infantum*, focusing on the role of T cell exhaustion. We used knockout mice for IDO (IDO^{-/-}), CD8 (CD8^{-/-}), and CD4 (CD4^{-/-}), as well as wild-type (WT) C57BL/6 mice as controls. After euthanasia, spleen, liver, and bone marrow were collected to evaluate parasite burden and immune parameters. Our results demonstrated that CD8^{-/-} mice exhibited a significantly higher parasite load in the bone marrow compared to WT animals. Flow cytometry analysis of splenocytes revealed a reduced frequency of PD-1-expressing CD4⁺ T cells in CD8^{-/-} and IDO^{-/-} mice, accompanied by increased IFN- γ production by CD4⁺ T cells in the CD8-deficient group. These findings suggest that PD-1 expression in CD4⁺ T cells may be modulated by the presence of CD8⁺ T cells.

Keywords: visceral leishmaniasis ;exhaustion ;proliferative capacity.

3367 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Non-canonical autophagy regulates host response in a murine model of *Legionella longbeachae*-induced pneumonia

MOREIRA, I.Z.; DOS SANTOS, M.D.R.Z.; MASCARENHAS, D.P.A.; CUNHA, L.D.. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Legionella longbeachae are Gram-negative intracellular bacteria and a leading cause of Legionnaires' disease. Unlike *L. pneumophila*, it induces mortality in mice due to severe lung immunopathology, despite cytokine-mediated control of bacterial replication. Here, we studied the participation of Rubicon (RUBCN), a component of a non-canonical pathway of recruitment of the autophagy machinery to the phagosome membrane, in the susceptibility to *L. longbeachae* infection. Our results indicate that global (*Rubcn*^{-/-}) or myeloid (*Rubcn*^{flox/flox}LysM-Cre⁺) ablation of RUBCN expression leads to higher susceptibility in a semi-lethal model of mice infection. Of note, the bacterial load in the lungs is not affected in *Rubcn*^{flox/flox}LysM-Cre⁺ mice. Instead, *Rubcn*^{flox/flox}LysM-Cre⁺ mice presented increased inflammatory infiltrate, as assessed by histological analyses and count of number of neutrophils in the bronchoalveolar lavage (BAL), more endothelial damage, determined by albumin dosing in the BAL, increased levels of IL-6 and IL-1 β and reduced levels of IL-10 in the lungs. Collectively, these preliminary findings are indicative that RUBCN depletion causes susceptibility associated with worsen lung damage during the acute phase of infection without affecting the resistance to bacteria. *L. longbeachae* infection drastically reduced the population of alveolar macrophages, while inducing an increase of the numbers of interstitial macrophages, compared to uninfected animals. While no differences in the frequency or numbers of these populations were observed between RUBCN-competent and -deficient animals, macrophages isolated from infected *Rubcn*^{flox/flox}LysM-Cre⁺ mice presented reduced expression of *Vegfa*, *Pla2g4a*, and *Mmp12*, suggesting that RUBCN may contribute directly to macrophage functional programming toward tissue repair *in situ*. Our results suggest that modulation of non-canonical autophagy may represent a potential therapeutic target to control infection-induced pathology

Keywords: *Legionella longbeachae*; pneumonia; non-canonical autophagy.

3368 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

TRM Marker Expression in Lung Infections: Peripheral Blood vs. Tracheal Aspirates

GONÇALVES, J.V.P.¹; DE MOURA, B.I.K.¹; DE ANDRADE, G.P.¹; GASSEN, R.B.¹; HAUBERT, A.F.¹; BONORINO, C.¹; SEADI, R.D.¹; MILESI, L.M.¹; HÖHER, J.A.²; DANIELLI, C.R.²; RODRIGUES JUNIOR, L.C.¹. 1. UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL; 2. HOSPITAL SANTA CLARA DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL.

Introduction: In the past decade, tissue-resident memory T cells (TRMs) have been recognized as a distinct subset of memory T lymphocytes retained long-term in non-lymphoid peripheral tissues, particularly at mucosal and epithelial barriers. Their strategic location and rapid effector functions enable robust protection against reinfections. While circulating lymphocytes expressing TRM-associated markers have been linked to favorable prognoses in lung cancer, their role during pulmonary infections is not well understood. **Objective:** To investigate whether acute pulmonary infections induce TRM-associated marker expression in peripheral blood and whether this correlates with clinical or immunological outcomes. **Methods:** Tracheal aspirates and peripheral blood were collected from four intubated patients with confirmed pulmonary infections, along with peripheral blood from five healthy individuals as controls. Samples underwent cellular isolation, staining, and flow cytometry using antibodies against CD3, CD4, CD8, CCR7, CD45RA, CD69, and CD103. **Results:** No TRM phenotype (CD69⁺CD103⁺) was detected among circulating T cells in either group. However, patients showed a reduction in circulating effector memory CD8⁺ T cells (CCR7⁻CD45RA⁺), possibly due to their recruitment and retention at infection sites, supported by increased TRM marker expression in tracheal aspirates. **Conclusion:** Peripheral blood apparently does not reflect TRM dynamics during pulmonary infections. These findings suggests the importance and the need of more tissue-specific analysis and further studies into the local immune response within infected lungs.

Keywords: Células TRM; Lung; Infection.

3369 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

A CRISPR based large-scale screening approach for the identification of relevant cellular pathways associated with encephalitogenic arbovirus infection

SILVA, B.M.S.¹; DAMASCENO, S.²; URBANSKI, A.H.³; GUERRA, R.O.⁴; RODRIGUES, T.S.⁵; MARTINS, R.B.⁶; ZAMBONI, D.S.⁴; SILVA, J.S.⁷; CUNHA, T.M.⁴; FRANÇA, R.F.D.O.⁷. 1. FIOCRUZ-SP, SÃO PAULO - SP - BRASIL; 2. RESEARCH CENTER FOR INFLAMMATORY DISEASES, RIBEIRÃO PRETO - SP - BRASIL; 3. CARLOS CHAGAS INSTITUTE, CURITIBA - PR - BRASIL; 4. FMRP-USP, RIBEIRÃO PRETO - SP - BRASIL; 5. UFSC, FLORIANÓPOLIS - SC - BRASIL; 6. FCFRP-USP, RIBEIRÃO PRETO - SP - BRASIL; 7. FIOCRUZ-SP, RIBEIRÃO PRETO - SP - BRASIL.

Arboviruses pose a serious public health threat due to the emergence of new viruses in susceptible populations, with a significant proportion of cases leading to severe neurological complications. Despite this, the molecular mechanisms that determine infection control versus progression to different clinical outcomes remain poorly understood. In this study, we employed a genome-wide CRISPR-Cas9 knockout approach using the Brunello library—comprising 77,441 guides targeting 19,000 human genes—to identify host factors involved in the response to arboviral infection in permissive cells. Following the generation of HuH-7 cell clones stably expressing the Cas9 enzyme (HuH7-Cas9), cells were transduced with the Brunello library. The resulting knockout cell pool was then subjected to sublethal and lethal infections with Oropouche (OROV) and Mayaro (MAYV) viruses. Next-generation sequencing of surviving cells post-infection revealed a set of genes and pathways associated with viral replication and virus-induced cell damage. Gene ranking and Gene Ontology (GO) analysis showed that these genes are enriched in key cellular processes. Based on these results, we selected a panel of biologically relevant genes for functional validation. For OROV-infected cells, the genes *HIPK3*, *IGF1R*, *PPP4R2*, *CASP5*, and *ADGRG5* were selected. For MAYV-infected cells, *TRIM8*, *OR5M10*, and *ASB18* were chosen. Specific guide RNAs (gRNAs) were designed for each gene to perform individual knockouts in HuH7-Cas9 cells. These single-gene knockout cells will be infected with OROV or MAYV and evaluated for viral load and cell viability at multiple time points post-infection. Here our findings validate the utility of CRISPR-Cas9 screening for uncovering novel molecular pathways involved in arboviral infection. This work contributes new insights into virus-host interaction mechanisms and may facilitate the identification of potential therapeutic targets.

Keywords: arboviruses ;cell damage ;CRISPR.

3372 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

A keratinocyte PKM2-dependent mechanism that fuels early cutaneous immunity and limits *Leishmania braziliensis* lesion growth

MENDES, M.T.¹; DE ASSUNÇÃO, S.F.V.¹; CHAGAS, G.G.¹; BENEVIDES, L.¹; DE CARVALHO, L.P.²; ALVES-FILHO, J.C.¹; SILVA, J.S.¹. 1. UNIVERSIDADE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. FICRUZ/BAHIA, SALVADOR - BA - BRASIL.

Cutaneous leishmaniasis caused by *Leishmania braziliensis* is characterized by ulcerative lesions and exaggerated inflammation. Keratinocytes are abundant skin cells that contribute to immune regulation during infection. The enzyme PKM2 regulates cellular metabolism and inflammatory responses. Here, we investigated the role of keratinocyte-specific PKM2 during *Leishmania braziliensis* infection using conditional knockout mice (K14^{creER2}Pkm2^{ff}). By the third week post-infection, K14^{creER2}Pkm2^{ff} mice developed larger lesions and had higher parasite loads than controls. RT-qPCR revealed reduced expression of inflammatory mediators, which was confirmed by a cytokine and chemokine PCR array showing downregulation of genes including *Adipoq*, *Bmp4*, *Bmp7*, *Ccl3*, *Ccl4*, *Ccl5*, *Ccl7*, *Cxcl10*, *Cxcl16*, *Il18*, *Il1a*, *Il1b*, *Il1rn*, *Mif*, *Pf4*, *Spp1*, *Vegfa*, and *Xcl1*. Flow cytometry showed decreased numbers of T lymphocytes, neutrophils, and monocytes in knockout mice. At five weeks post-infection, knockout mice displayed increased IFN- γ and IL-4, indicating delayed inflammation; however, parasite burden and lesion severity remained high, with persistently low iNOS expression suggesting impaired macrophage activation. Analysis of public RNA-seq data from human cutaneous leishmaniasis lesions revealed elevated PKM2 expression compared to healthy skin, supporting translational relevance. Our data indicate keratinocyte-intrinsic PKM2 is essential for orchestrating inflammatory mediator production, immune cell recruitment, and effective immunity against *L. braziliensis*, highlighting an immunoregulatory role of keratinocytes mediated via metabolic pathways.

Keywords: PKM2, keratinocytes, cutaneous leishmaniasis; *Leishmania braziliensis*; cytokines, immune regulation.

3375 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

IL-27 and Type I IFNs Modulate Pulmonary Immunity and Susceptibility During Cryptococcal Infection

NETO, A.C.D.O.¹; BOCCA, A.L.²; DE SOUZA, R.L.L.²; BASSI, V.²; BENEVIDES, L.²; SILVA, J.S.². 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. PLATAFORMA BI-INSTITUCIONAL DE PESQUISA EM MEDICINA TRANSLACIONAL, FIOCRUZ-SP., RIBEIRÃO PRETO - SP - BRASIL.

Cryptococcus neoformans is an opportunistic fungus that causes severe meningoencephalitis, particularly in immunocompromised individuals. Considering the importance of the immune response in containing this infection, this study aimed to investigate the immunomodulatory roles of interleukin-27 (IL-27) and type I interferon (IFN-I) during pulmonary cryptococcosis. To this end, wild-type mice, IL-27 knockout (IL-27 KO) mice, and IFNAR knockout (IFNAR KO) mice were infected intratracheally with 10^5 *C. neoformans* cells, and we evaluated cell migration to lung tissue by flow cytometry, histopathology, and fungal load (CFU) at 7, 14, and 21 days post-infection (d.p.i.). In our preliminary results, WT mice had higher fungal loads at 14 d.p.i., suggesting that absence of IL-27 or IFN-I may favor more effective initial fungal control. However, at 21 d.p.i., all groups showed intense pulmonary colonization. Histological analysis of lung tissue revealed more intense inflammation and tissue damage in WT animals, while knockouts showed less lung damage despite similar fungal loads. Furthermore, we observed that lymphoid cell expansion in WT mice was delayed, peaking at 21 d.p.i., while IL-27 KO mice showed recruitment of this cell type at 14 days post-infection. Regarding myeloid cells, especially macrophages (CD11b+F4/80+), in WT mice, it was more pronounced at 14 days post-infection, suggesting dependence on IL-27 and IFN-I signaling. No significant differences were found between groups in CD11c+ MHCII+, CD4+, or CD8+ populations. Functionally, IFN- γ production by CD4+ T cells increased significantly only in IFNAR KO animals at 14 d.p.i., while IL-17 levels increased in all groups at 21 d.p.i., especially in knockouts, indicating a possible compensatory immune mechanism. These findings suggest IL-27 and IFN-I critically regulate immune cell dynamics and pulmonary inflammation during cryptococcal infection, potentially influencing host susceptibility and disease progression.

Keywords: *Cryptococcus neoformans*; Immunomodulation; Knockout mice (KO mice).

3376 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Impact of PI3K δ -Mediated Signaling on the Progression of *Trypanosoma cruzi* Infection

ALVES, C.F.¹; DA SILVA, M.C.²; RIGONI, T.D.S.³; SOARES, H.A.D.S.³; NONATO, O.C.D.S.³; BENEVIDES, L.³; GARDINASSI, L.G.A.⁴; CUNHA, T.M.⁴; SILVA, J.S.³. 1. FIOCRUZ/SP, RIBEIRÃO PRETO - SP - BRASIL; 2. FEDERAL UNIVERSITY OF ALAGOAS- UFAL, MACEIO - AL - BRASIL; 3. FIOCRUZ/USP, RIBEIRÃO PRETO - SP - BRASIL; 4. USP, RIBEIRÃO PRETO - SP - BRASIL.

Introduction: Phosphatidylinositol 3-kinase delta (PI3K δ) is predominantly expressed in hematopoietic cells and plays a main role in immune regulation. Previous studies have shown that PI3K δ inactivation may confer either resistance or susceptibility depending on the pathogen involved. Our group previously demonstrated the role of the PI3K γ isoform during *Trypanosoma cruzi* infection, showing that its absence impairs myeloid cell function, leading to exacerbated myocarditis and increased mortality in mice. However, the specific contribution of PI3K δ during *T. cruzi* infection remains unexplored. To address this, we aim to investigate the mechanisms by which PI3K δ influences host resistance or susceptibility. **Methods:** Mutant mice PI3K $\delta^{D910A/D910A}$ were infected with 10^3 trypomastigotes of the Y strain of *T. cruzi*. Survival and body weight were monitored over 20 days. Heart and liver parasitism was assessed by PCR-based detection of parasite DNA. Parasite-specific antibody levels were quantified by ELISA using serum samples, which were also subjected to biochemical analyses. CD4+ and CD8+ T cell responses were evaluated by flow cytometry. **Results:** We observed that mutant mice exhibited more susceptibility to infection, as evidenced by increased parasitemia, heart and liver parasitism, pronounced tissue damage, weight loss, and mortality. These mice displayed reduced levels of parasite-specific antibodies, along with reduced expression of perforin, granzyme, and Th1-associated cytokines in cardiac tissue. Flow cytometric analysis of splenic tissue revealed increased Live/Dead staining in CD3+ T cells and a reduced frequency and granzyme production in CD8+ T cells. **Conclusion:** Our findings demonstrate that PI3K δ is indispensable for the development of a robust immune response against *T. cruzi*. This isoform plays a key role in promoting both cytotoxic and humoral immunity, and appears to be important for signaling pathways that support T cell survival during infection.

Keywords: Phosphatidylinositol 3-kinase delta; T; cruzi; T cell.

3377 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

CD8+bright and CD8+dim T Cell Imbalance Reflects Clinical Severity in Leprosy

GAMA, A.L.P.R.¹; DE CASTRO, Y.S.²; DA SILVA, J.A.²; NASCIMENTO, L.S.²; NAHN JUNIOR, E.P.³. 1. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO - UENF, CAMPOS DOS GOYTACAZES - RJ - BRASIL; 2. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO, CAMPOS DOS GOYTACAZES - RJ - BRASIL; 3. FACULDADE DE MEDICINA DE CAMPOS, CAMPOS DOS GOYTACAZES - RJ - BRASIL.

Leprosy, caused by *Mycobacterium leprae*, manifests across a clinical spectrum influenced by the host's immune response from a strong, protective cellular response in tuberculoid forms to a weak cellular response in lepromatous forms. CD8+ T lymphocytes play a critical role in defense against intracellular pathogens. In this study, we analyzed CD3+ CD45+ CD8+ T cells in 91 individuals (22 healthy controls and 69 leprosy patients with several clinical forms), using flow cytometry for extracellular and intracellular staining for cells, cytokines and cytotoxic granules identification. Although no significant differences were observed in total CD8+ T cell percentages, two distinct subpopulations were identified based on fluorescence intensity: CD8+/bright and CD8+/dim T cells. Multibacillary patients exhibited a marked reduction in CD8+/bright cells compared to paucibacillary patients and healthy controls. Similarly, according to the Madrid classification, individuals with the most severe clinical forms (borderline and lepromatous) showed decreased CD8+/bright and increased CD8+/dim cells compared to those with indeterminate or tuberculoid forms and healthy controls. CD8+/dim cells had lower production of TNF- α , IFN- γ , and IL-4, while CD8+/bright cells exhibited low IL-4 and IL-10 expression. Granzymes and perforin production were primarily associated with CD8+/dim T cells, suggesting their cytotoxic activity, whereas CD8+/bright T cells showed reduced cytotoxic potential. These findings reveal, for the first time, distinct functional roles for CD8+/bright and CD8+/dim T cells in leprosy immunopathogenesis. This study reveals a functional imbalance between CD8+ T cell subpopulations in leprosy, highlighting a potential role for CD8+bright and CD8+dim cells in modulating disease severity. These findings contribute to a better understanding of cellular immune responses in leprosy and may inform future immunotherapeutic strategies.

Keywords: Leprosy; Immune Response; CD8 T lymphocytes.

3382 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Evaluation of the Inflammatory Response in PASC (Long COVID) Patients

CORREA, F.C.; FERREIRA, T.R.; DEZEN, A.L.O.; DIAS-MELICIO, L.A.. UNESP, BOTUCATU - SP - BRASIL.

Long COVID is a complex and debilitating condition that occurs in at least 10% of individuals infected with SARS-CoV-2. Despite the estimated 65 million cases, it remains a poorly characterized disease, both clinically and etiologically. A significant challenge in its study is the considerable overlap with other conditions, making it exceedingly difficult to differentiate cases of Long COVID from cases exhibiting persistent symptoms due to physical sequelae rather than potential chronic viral infection. By meticulously selecting patients, the objective of this study was to assess aspects of immunity in individuals with Long COVID and compare them to those who had COVID-19 and did not develop sequelae. Our results showed that patients, in addition to having increased expression of TLR4 in B lymphocytes, also presented with decrease of at least half of the population of CD4+ T lymphocytes and an increase in the population of CD8+ T lymphocytes. We also found an absolute increase in the population of non-classical monocytes and a significant loss in the expression of ACE2 on the membrane of classical monocytes. Moreover, we characterized an increase in IL-6 and IL-17A in patients, and higher values of IL-4, IL-2, IL-1 β , TNF- α , IL-10, and IL-30 12p70 in controls. Therefore, there is evidence consistent with current literature, showing a possible chronic inflammatory response with a decrease in CD4+ T cells and an increase in non-classical monocytes, possibly in response to viral antigen reservoirs present in the body.

Keywords: SARS-CoV-2;(PASC) Long COVID;cytokines.

3390 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The proinflammatory toxin ExoU impairs host antioxidant response by targeting Nrf2 during *Pseudomonas aeruginosa* infection

EMMERICK, K.R.; SEQUEIRA, Y.D.C.; SALIBA, A.M.. STATE UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Pseudomonas aeruginosa is a highly adaptable Gram-negative opportunistic bacterium frequently associated with potentially severe infections, particularly in immunocompromised individuals. Equipped with multiple virulence factors, *P. aeruginosa* can directly inject the ExoU toxin, a highly cytolytic phospholipase, into the host cell cytosol, rapidly leading to cell death by targeting the host membrane. In addition to its cytolytic capacity, ExoU amplifies cell death by contributing to oxidative stress as a result of its pro-inflammatory activity and interference with the host's antioxidant response. To better understand the impact of ExoU on the host's antioxidant defense, this study aimed to evaluate whether this toxin modulates the expression of Nrf2 and HO-1, two genes associated with the antioxidant response, during *P. aeruginosa* infection. For this purpose, human lung carcinoma epithelial cells (A549 cell line) were infected with MOI 20 or MOI 100 of the ExoU-producing strain PA103 and its isogenic mutant PA103 Δ exoU, lacking the toxin-encoding gene. The infection lasted 1 hour, followed by optional gentamicin treatment for an additional 17 hours to eliminate extracellular microorganisms. ExoU cytotoxicity after 18 hours was confirmed using MTT reduction assays, while Nrf2 and HO-1 gene expression was evaluated at 1 and 18 hours by RT-qPCR. The results demonstrated that ExoU inhibits Nrf2 expression in a time- and dose-dependent manner, compromising the host cells' ability to counteract oxidative stress, increasing cellular damage and favoring infection progression. Interestingly, no significant statistical differences were observed between the two strains in HO-1 expression. Thus, this study contributed to advancing the understanding of ExoU virulence mechanisms and suggests that positive modulation of Nrf2 could offer new therapeutic strategies to combat infections caused by multidrug-resistant *P. aeruginosa* strains.

Keywords: *Pseudomonas aeruginosa* gene expression; ExoU; Nrf2.

3393 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Modulation of purinergic signaling in COVID-19

BARBOSA, N.C.¹; SAVIO, L.E.B.¹; DUTRA, L.S.¹; DA CRUZ, L.D.O.¹; TEMEROZO, J.²; KURTENBACH, E.¹; TAKIYA, C.M.¹; BOU-HABIB, D.C.²; COUTINHO-SILVA, R.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Purinergic signaling may act either detrimentally or beneficially in infectious diseases, assuming a significant and sometimes pivotal role in their pathophysiology. In the context of viral infections, for instance, ATP is released into the extracellular environment. Our recent evidence indicates that SARS-CoV-2 infection leads to elevated circulating ATP levels, supporting the idea that this danger-associated signal may drive disease progression. The P2X7 receptor, an ion channel activated by extracellular ATP (eATP), is known to trigger pro-inflammatory responses. Therefore, our aim was to investigate the role of ATP-P2X7 signaling in the immune response to SARS-CoV-2 using transgenic K18-hACE2 mice. To inhibit the P2X7 receptor, 50 mg/kg of Brilliant Blue G (BBG) was administered intraperitoneally one day before SARS-CoV-2 inoculation (2×10^4 PFU, intranasally). The animals were divided into four groups: MOCK, MOCK + BBG, SARS, and SARS + BBG ($n = 3-7$). After seven days, the animals were euthanized, and lungs and blood were collected for analysis. mRNA levels of P2X7, CD39, IL-1 β , and P2Y12 were analyzed by RT-qPCR. Plasma was obtained to determine circulating levels of eATP, LDH, and cytokines. We observed a decrease in body weight (MOCK vs. SARS) and increased mortality (MOCK vs. SARS and SARS vs. SARS + BBG) from the fourth day of infection onward. There was a significant increase in circulating eATP, LDH, IL-1 β , and IFN- γ levels in infected animals (MOCK vs. SARS), which were reduced upon BBG treatment. A significant increase in CD39, IL-1 β , IL-6, IFN- γ , and P2Y12 mRNA levels was observed in the lungs of the SARS group vs. MOCK. Histological analysis of lung tissue revealed severe lesions in the SARS group compared to MOCK. Our findings demonstrate that the K18-hACE2 mouse model is a robust experimental tool for studying SARS-CoV-2 infection and indicate that purinergic signaling is significantly modulated in this pathological context. Funding: FAPERJ, CNPq.

Keywords: ATP; SARS-COV-2; K18-ACE-2.

3398 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

EVALUATION OF OXIDATIVE STRESS IN THE SERUM OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS IN NORTHERN MATO GROSSO

OLIVEIRA, E.L.D.S.¹; FRANÇA, C.G.M.¹; BARBOZA, F.²; SINHORIN, V.D.G.³; ALBIERO, L.R.³. 1. ATENAS, SORRISO - MT - BRASIL; 2. CIMED CLINIC, SINOP - MT - BRASIL; 3. UFMT, SINOP - MT - BRASIL.

Introduction: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease resulting from delayed clearance of apoptotic bodies. Oxidative stress plays a fundamental role in SLE's inflammatory process, arising from the imbalance between free radical production and antioxidant neutralization, leading to tissue damage. **Objective:** This study aimed to evaluate in vitro the oxidative stress in patients with SLE from northern Mato Grosso. **Methodology:** The following biomarkers were assessed: Reduced Glutathione (GSH), Vitamin C, Carbonylated Proteins (Carbonyl), Thiobarbituric Acid Reactive Substances (TBARS), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione S-transferase (GST), and Glutathione Peroxidase (GPx). The experimental group consisted of 9 patients with active SLE using only 400 mg of Hydroxychloroquine, and the control group consisted of 9 healthy, medication-free individuals without comorbidities. Groups were matched for age, sex, and immunogenic profile. Statistical analysis was performed using the T-test or Mann-Whitney test, with a significance level of $p < 0.05$ (CEP/UFMT/Sinop Approval: 6.284.005). **Results:** Non-enzymatic antioxidants and oxidative stress markers showed no significant differences between the SLE and control groups. However, the enzymatic antioxidant CAT showed a significant increase in the serum of SLE patients compared to controls. **Conclusion:** This study analyzed oxidative stress parameters in the serum of SLE patients from northern Mato Grosso and compared them with healthy individuals. Although the CAT biomarker activity increased, other parameters showed no significant differences, suggesting that this response pattern may be region-specific or require a larger sample size for confirmation.

Keywords: Systemic Lupus Erythematosus;Oxidative stress;Autoimmunity;.

3403 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

IL-8-Secreting Senescent B Cells as Novel Contributors to the Inflammatory Milieu in Localized Cutaneous Leishmaniasis

RIBEIRO JUNIOR, R.S.¹; DE MOURA, R.G.¹; COVRE, L.P.¹; LOPES, P.O.¹; FARDIN, J.M.¹; FALQUETO, A.¹; DECOTE-RICARDO, D.²; FREIRE-DE-LIMA, C.G.³; GUEDES, H.L.D.M.³; MARTINS, A.M.D.F.⁴; AKBAR, A.⁵. 1. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 2. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO,, RIO DE JANEIRO - RJ - BRASIL; 4. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL; 5. UNIVERSITY COLLEGE LONDON, LONDON - REINO UNIDO.

Leishmaniasis is a neglected tropical disease caused by protozoa of the Leishmania genus and transmitted through the bite of infected sandflies. Among its clinical forms, localized cutaneous leishmaniasis (LCL) is the most prevalent, characterized by ulcerated skin lesions at the site of parasite inoculation. The host immune response in LCL plays a dual role, since it is essential for parasite control but can also drive tissue damage and chronic inflammation. Recently, our group have evidenced that senescent immune cells, particularly T and NK lymphocytes, are enriched in LCL lesions and are associated with poor clinical outcomes. Cellular senescence is marked by telomere shortening, irreversible cell cycle arrest, and acquisition of the senescence-associated secretory phenotype (SASP), which includes the production of pro-inflammatory mediators such as IL-8. Although the role of B lymphocytes in LCL pathogenesis remains incompletely understood, they have been suggested to exert immunomodulatory effects, primarily through anti-inflammatory cytokines like IL-10. In this study, we used immunofluorescence to demonstrate that B cells within LCL lesions can acquire a senescent phenotype, as evidenced by p16 expression. Furthermore, we identified CD19⁺ B cells capable of secreting IL-8, indicating their potential contribution to local inflammation. Notably, for the first time, we detected a rare CD19⁺CD3⁺ B cell subset in the skin of LCL patients. These cells appear to undergo senescence within the lesion microenvironment and may influence the immunopathology through SASP-related mechanisms. Our findings suggest a novel role for senescent B cells in the inflammatory milieu of LCL lesions, though further studies are required to elucidate their functional relevance and potential as therapeutic targets.

Keywords: B Cells;Localized Cutaneous Leishmaniasis;senescence.

3404 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Profile of untargeted metabolome in chronic Chagas cardiomyopathy patients and its association with immune cell metabolism

BONECINI, G.¹; RENZETTI, A.R.D.S.¹; DE SOUZA, H.R.²; DE LIMA, E.H.R.¹; DE ARAUJO, F.M.D.S.¹; MAIA, R.M.³; LAMOGLIA, A.¹; PORTELA, L.F.¹; FERREIRA, R.R.¹; BRITO, P.D.¹; DA SILVA, P.S.¹; QUINTANA, M.S.¹; SANGENIS, L.H.¹; DE HOLANDA, M.T.⁴; HASSLOCHER-MORENO, A.M.¹; DE ARAÚJO-JORGE, T.C.⁵; LANNES-VIEIRA, J.⁵; DA COSTA, R.G.²; SARAIVA, R.M.⁶. 1. INI/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. LABMETA/UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. INI/BRAZIL, RIO DE JANEIRO - RJ - BRASIL; 4. INI/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 5. IOC/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 6. INI/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

It is estimated that Chagas disease (CD) affects more than 6 million individuals around the world. Dysfunctions in energy metabolism play a role in the development of cardiovascular diseases and are associated with immune cell function. Metabolomics has been used to identify molecular targets that may be associated as biomarkers for infectious diseases outcome. We performed an untargeted metabolome assay from 150 CD patients and 50 health control (HC) to identify serum metabolites as potential biomarkers for CCC progression. Serum liquid chromatography analysis was submitted to a metabolomics workflow using MS-DIAL software. Metabolic pathways map was built using the MetaboAnalyst platform. Two data matrices containing 1770 and 2230 features for positive and negative poles were identified. Fifteen candidate biomarkers were identified. Those which were significantly higher in the CD group, include adenine, cysteine, glutamine and inosine, that are crucial for the activation and function of T cells, macrophages, and neutrophils, by its involvement in protein degradation, antigen presentation, cell signaling, energy source for immune cells and cytokines production. Arachidonic acid, 1-Palmitoyl, LysoPE, glutamic acid, hypoxanthine, palmitic acid, leu-leu, phenylalanine, phe-phe, serine and xanthine were notably decreased in CD group. Those are involved in the production of inflammatory mediators (prostaglandin, thromboxane, and leukotriene), neutrophil function, cell adhesion and migration, apoptosis, macrophages polarization and cytokines production. Significant metabolic pathways were glycine, serine and threonine metabolism; glyoxylate and dicarboxylate metabolism; alanine, aspartate and glutamate metabolism; phenylalanine, tyrosine and tryptophan biosynthesis; and glutathione metabolism. These results in a novel exploratory study identified potential biomarker candidates that can be used in clinical practice, enhancing patients' survival and quality of life.

Keywords: METABOLOME;CHAGAS DISEASE;IMMUNE- RELATED METABOLISM.

3405 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Impact of P2RX7 rs3751143A>C Polymorphism on Chemokine Profiles in Leprosy

SOUZA, R.D.C.¹; CLEN, R.R.S.¹; RAQUEL, L.L.S.¹; DA SILVA, J.A.¹; NAHN JUNIOR, E.P.²; GAMA, A.L.P.R.¹. 1. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO, CAMPOS DOS GOYTACAZES - RJ - BRASIL; 2. FACULDADE DE MEDICINA DE CAMPOS, CAMPOS DOS GOYTACAZES - RJ - BRASIL.

Leprosy, caused by *Mycobacterium leprae*, presents clinical manifestations influenced by the host immune response. The P2X7 receptor, encoded by *P2RX7*, promotes inflammasome activation, cytokine and chemokine release, and the death of infected macrophages. Chemokines act by recruiting leukocytes to the infection site, contributing to the local inflammatory profile. Single nucleotide polymorphisms (SNPs) in *P2RX7* may modulate the disease's immunopathology. The SNP rs3751143 A>C, a loss-of-function variant, was associated with susceptibility to leprosy, but its effect on immunological parameters remains unclear. We evaluated serum levels of RANTES, MCP-1, IL-8, MIG, and IP-10 using Cytometric Bead Array and assessed the influence of the SNP on their levels in 22 controls and 20 leprosy patients. Controls showed increased levels of RANTES ($p<0.001$) and IP-10, while patients exhibited higher levels of MIG ($p<0.05$), MCP-1 and IL-8, suggesting opposing production patterns between the groups. Clinical form analysis showed a trend toward higher levels of RANTES, MIG, and IP-10 in multibacillary patients, with statistical significance only for IP-10 ($p<0.05$). Regarding genotypes, regardless of disease status, CC individuals showed a trend toward higher production of RANTES, MCP-1, IL-8 and MIG, although without statistical significance. In contrast, AA individuals exhibited greater IP-10 production ($p<0.05$). In the genotype analysis considering both controls and patients, RANTES production was higher in CC controls compared to AA patients ($p<0.05$). Moreover, the presence of the C allele in controls was also associated with higher RANTES levels when compared to the AA group among patients ($p<0.05$). Controls with the AA genotype showed significantly higher IP-10 levels than AA patients ($p<0.05$). These data suggest that individuals with leprosy have reduced production of RANTES and IP-10, but the CC genotype may attenuate this reduction by modulating chemokine production.

Keywords: Leprosy;rs3751143A>C;Chemokines.

3409 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

CYTOKINE PROFILE IN PATIENTS WITH DENGUE, CHIKUNGUNYA OR OROPOUCHE VIRUSES

PIMENTA, B.C.¹; SARMENTO, I.V.¹; ZORDAN, J.S.N.¹; TAVARES, E.A.²; GOULART, J.P.²; NODARI, J.Z.²; CÔ, A.C.G.²; DE AZEVEDO, S.S.D.²; DELATORRE, E.O.¹; RODRIGUES, R.R.²; COVRE, L.P.³; GOMES, D.C.D.O.¹. 1. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 2. LABORATÓRIO CENTRAL DE SAÚDE PÚBLICA DO ESTADO DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 3. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, VITÓRIA - ES - BRASIL.

Arboviral infections remain critical public health concerns in Latin America due to the increasing incidence, severity, and geographic expansion of dengue virus (DENV), chikungunya virus (CHIKV), and more recently, Oropouche virus (OROV). Despite being transmitted by different vectors, these infections share overlapping clinical symptoms and provoke strong immune responses characterized by elevated pro-inflammatory cytokine production. Such an inflammatory milieu may compromise antiviral immunity by fostering dysfunctional or senescent immune cell phenotypes, potentially contributing to disease severity. This study aimed to characterize the inflammatory cytokine profiles of patients infected with DENV, CHIKV, or OROV. After approval by the Institutional Human Research Ethics Committee (protocol 84698324.7.0000.5064), acute-phase serum samples from 89 patients (29 DENV, 30 CHIKV, 30 OROV) confirmed by RT-qPCR were analyzed. Cytokine levels were measured using the LEGENDPlex™ Human Inflammation Panel 1 via Cytokine Bead Assay (CBA), with data acquired through multiparameter flow cytometry and analyzed using BioLegend software. CHIKV-infected patients exhibited significantly higher levels of IFN- α 2, TNF- α , MCP-1, IL-6, and IL-12p70 compared to OROV, while IL-23 was reduced. In DENV cases, TNF- α , IL-10, and IL-12p70 were elevated relative to OROV, and IL-23 was increased compared to CHIKV. CHIKV patients also showed elevated MCP-1, IL-6, and IL-17A versus DENV. Across all infections, IFN- α 2, MCP-1, IL-8, and IL-18 were significantly increased when compared to healthy controls (HC). CHIKV also showed specific increases in IL-6, whereas IL-12p70 and IL-23 were higher in HC than CHIKV. OROV patients exhibited reduced IL-12p70 compared to HC. No significant differences were observed in IL-1 β , IFN- γ , or IL-33 levels. These results indicate distinct cytokine signatures among arboviral infections, with CHIKV eliciting the most pronounced pro-inflammatory response.

Keywords: Arboviruses;Cytokine;Inflammation.

3417 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Bone marrow impairment and its relationship with the pathogenesis of Mayaro virus infection

VARGES, V.C.¹; DE OLIVEIRA, M.A.¹; REAL, F.R.O.²; DA POIAN, A.T.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUT PASTEUR DE LILLE, LILLE - FRANÇA.

The Mayaro virus (MAYV), transmitted by *Haemagogus janthinomys* mosquitoes, causes a disease similar to Chikungunya fever, with symptoms like fever, headache, muscle, and joint pain. Thrombocytopenia and lymphopenia are common among patients. MAYV initially replicates in lymph nodes, liver, and spleen, then reaches muscles and joints, potentially causing chronic inflammation. While joint inflammation and bone loss are known following MAYV infection, its effect on bone marrow remains unclear. The bone marrow is the primary site of adult hematopoiesis. However, if damaged, the liver and spleen can resume this function. How arthritogenic alphavirus infections impact hematopoiesis and relate to viral pathogenesis is poorly understood. This study investigates potential bone marrow damage from MAYV infection, exploring its relationship to hematological changes and disease pathogenesis, using a murine model that mimics human disease. SV129 mice were infected with MAYV and euthanized at various times for blood and tissue collection. These samples were used for qPCR, histological, and hematological analyses. Preliminary results revealed tissue damage and fibrosis in the bone marrow of infected animals, indicating hematopoietic compromise. Supporting these findings, reticulocytopenia, lymphocytosis, and increased red blood cell size variation were observed. A reduced growth plate area in tibias also suggested an effect on bone development. The spleen showed increased cell density. Liver histological analysis revealed hepatocyte swelling and myeloid progenitor cells, aligning with possible hematopoietic disruption. The presence of megakaryocytes in the liver and viral RNA within them confirms direct viral action. Therefore, our findings indicate a significant impact on hematopoiesis during MAYV infection.

Keywords: Mayaro virus;hematopoiesis;alphavirus.

3418 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Capsular polysaccharides from *Cryptococcus neoformans* can modulates in vitro *Trypanosoma cruzi* infection

LIANI, S.A.C.¹; DE OLIVEIRA, J.C.G.¹; LIMA, I.D.¹; GOMES, A.I.N.¹; CAVALCANTI, E.J.O.¹; DE ALMEIDA, G.J.M.¹; CARDOSO, M.F.²; COVRE, L.P.³; DA SILVA-JUNIOR, E.B.¹; PREVIATO, L.M.¹; PREVIATO, J.O.¹; DECOTE-RICARDO, D.⁴; FREIRE-DE-LIMA, C.G.¹. 1. IBCCF/UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. IFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. UFES, VITÓRIA - ES - BRASIL; 4. UFRRJ, RIO DE JANEIRO - RJ - BRASIL.

Cryptococcus neoformans is a basidiomycete opportunistic fungal pathogen with a global distribution and is the causative agent of cryptococcosis, a disease responsible for 200.000 deaths annually. The infection typically begins in the lungs, but the meningoencephalitis is the most severe form of the disease and potentially results in death. Biochemical studies have shown that the fungal capsule is predominantly composed of glucuronoxylomannan (GXM), followed by glucuronoxylomannogalactan (GXMGal) and mannoproteins. Capsular polysaccharides have been reported to exhibit distinct immunomodulatory effects: while GXM predominantly exerts immunosuppressive activity, inhibiting immune responses, GXMGal has been associated with immunoprotective properties. To evaluate these differential immunomodulatory effects in *Trypanosoma cruzi* infection model, we employed an in vitro system using murine macrophages infected with the *T. cruzi* DM28c strain, followed by treatment with either GXM or GXMGal. Our data demonstrated that at 7-10 dpi, the number of trypomastigotes released was higher in GXM-treated conditions compared to GXMGal treated, even in the presence of interferon- γ . Additionally, amastigote counts were higher in macrophages treated with GXM, whereas GXMGal-treated conditions showed increased nitric oxide production. Cytokine quantification revealed elevated TGF- β levels in GXM-treated cells and increased TNF- α levels in GXMGal treated conditions. Furthermore, inhibition assays confirmed that these effects were reversed upon treatment with specific inhibitors. In vitro infection and treatment model confirmed that the immunomodulatory effects of *C. neoformans* capsular components persist even in a distinct infection setting. These findings reinforce the role of GXM as an immunosuppressive factor and GXMGal as an immunoprotective agent, suggesting that capsular components may differentially modulate host immune responses in different infectious contexts.

Keywords: *Cryptococcus neoformans*; Capsular polysaccharides; *Trypanosoma cruzi*.

3427 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Analysis of Growth Differences Between *Sporothrix brasiliensis* and *Sporothrix schenckii* and Their Interaction with feline macrophage cell line.

SANTANA, M.M.F.¹; VIEIRA, W.W.D.S.²; FREIRE-DE-LIMA, C.G.²; NASCIMENTO, D.D.O.¹. 1. UFRRJ, SEROPEDICA - RJ - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Sporotrichosis is a subacute-to-chronic mycosis considered a neglected disease by the World Health Organization (WHO). It affects humans and various animal species and is caused by fungi belonging to the pathogenic clade of the genus *Sporothrix*. In the ongoing zoonotic epidemic in Brazil, *Sporothrix brasiliensis* is the most prevalent species, with transmission mainly associated with contact with infected cats. This zoonotic transmission pattern has been linked to more severe clinical presentations in humans, including increased reports of serious cases in immunocompetent individuals. Despite the disease's importance, little is known about the immune response in feline infection. To address this, we initially evaluated the growth of *Sporothrix* isolates under different glucose supply conditions. A glucose dose-dependent growth pattern was observed, with *S. schenckii* showing distinct behavior compared to *S. brasiliensis* isolates. In vivo infection was conducted using *Galleria mellonella* larvae, and a higher mortality rate was observed in the group infected with *S. brasiliensis*. To better understand these differences, we also performed infection in Fcwf-4 feline macrophage cell line, where cultures containing 10⁵ cells were infected with yeast cells at a 5:1 ratio. After 24 hours of infection, no cell death was observed, nor were there differences in nitric oxide (NO) production or in the number of phagocytosed yeast cells. Additional in vitro experiments are ongoing to investigate the mechanisms underlying the higher in vivo infectivity observed in *S. brasiliensis* compared to *S. schenckii* yeast cells.

Keywords: Sporotrichosis; *Sporothrix* sp; Fcwf-4 feline macrophage cell lines.

3432 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Role of the *Schistosoma mansoni* Sm29 antigen in modulating *Leishmania braziliensis* infection

NETO, P.P.D.O.¹; SANTANA, J.B.¹; AZEVEDO, S.B.¹; DE PASCALI, D.J.¹; MACHADO, P.R.¹; DE CARVALHO FILHO, E.M.²; CARDOSO, L.S.¹. 1. SERVIÇO DE IMUNOLOGIA, HOSPITAL UNIVERSITÁRIO PROFESSOR EDGARD SANTOS, UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. LABORATÓRIO DE PESQUISAS CLÍNICAS, INSTITUTO GONÇALO MUNIZ – FIOCRUZ, INSTITUTO NACIONAL DE CIÊNCIA E TECNOLOGIA EM DOENÇAS TROPICAIS, INCT-DT/CNPQ,, SALVADOR - BA - BRASIL.

Cutaneous leishmaniasis presents a wide range of clinical manifestations and is caused by protozoa of the *Leishmania* genus. The immune response is predominantly Th1/inflammatory, activating macrophages to release cytokines and pro-inflammatory molecules that mediate parasite elimination. However, when excessive, this response contributes to disease pathogenesis. Conventional therapy is often associated with treatment failures and adverse side effects, highlighting the need for alternative therapeutic strategies. In this context, helminth antigens, such as Sm29, have been investigated for their immunoregulatory potential. The aim of this study was to evaluate the immunological effects of the nanocomposite Sm29/Au in the regulation of macrophages and peripheral blood mononuclear cells (PBMCs) infected with *L. braziliensis* in vitro. The study was approved by the UFMG Ethics Committee (approval number 3.002.786). PBMCs and monocyte-derived macrophages from healthy individuals were isolated and infected with *L. braziliensis* promastigotes (5:1), then stimulated for 24 hours with Sm29 antigen encapsulated in gold nanorods (Sm29/Au) and isolated Sm29 antigen. Levels of IL-10, IFN- γ , and IL-1 β were measured by ELISA; nitric oxide (NO) production was assessed using the Griess method; macrophage infectivity was evaluated by light microscopy; and expression of arginase, iNOS, and TGF- β was analyzed by RT-PCR. Stimulation with Sm29/Au and Sm29 increased IL-10 production in PBMC and macrophage supernatants. There was no change in IFN- γ levels in PBMCs. Stimulation with Sm29 did not induce significant changes in infectivity, expression of arginase, iNOS, and TGF- β , or in NO production, suggesting that despite its immunoregulatory potential, this antigen did not alter the leishmanicidal mechanisms of effector cells.

Keywords: *Leishmania braziliensis*; Macrophages; Sm29.

3434 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

THE ABSENCE OF THE B1 RECEPTOR IS ASSOCIATED WITH MICE PROTECTION AGAINST *Pseudomonas aeruginosa* INFECTION

SILVA, M.E.F.¹; LUCAS, L.M.B.¹; BARBOSA, J.P.P.¹; AZEVEDO, L.M.S.¹; AMARAL, B.S.¹; OLIVEIRA, S.N.¹; SANTOS, B.M.D.A.¹; QUEIROZ JUNIOR, C.¹; ARIFA, R.D.D.N.²; DE SOUZA, D.D.G.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), BELO HORIZONTE - MG - BRASIL.

Pseudomonas aeruginosa is an opportunistic pathogen multidrug-resistant bacteria able to cause acute or chronic infections in immunosuppressed individuals with cystic fibrosis, cancer, sepsis, or ventilator-associated pneumonia. Its infectivity is attributed to virulence factors, including lipopolysaccharides, outer membrane proteins, flagella, and toxins secreted through secretion systems. These virulence factors trigger the release of a series of pro-inflammatory mediators, among which are bradykinin and des-Arg(9)-bradykinin. Des-Arg(9)-bradykinin is a nanopeptide formed from bradykinin metabolism and binds to B1 receptor. This receptor is expressed under inflammation, tissue injury, or bacterial threat, making it a target for studying inflammatory diseases. Therefore, our project aims to evaluate the role of B1 receptor activation in the murine model of *Pseudomonas aeruginosa* infection. For this purpose, C57BL/6 WT and *Bdkrb1*^{-/-} mice (CEUA:307/2023) were infected intranasally with 10⁷ of PAK, a strain of *P.aeruginosa*. WT group had a lethality of 83% within five days, while *Bdkrb1*^{-/-} mice displayed 100% of survival. Subsequent experiments were conducted 24 hours post-infection, with both WT and *Bdkrb1*^{-/-} animals euthanized for bronchoalveolar lavage, lung and liver evaluation. Our results showed that bacterial recovery was lower in *Bdkrb1*^{-/-} compared to WT. In an interconnected way, the total number of leukocytes and neutrophils after infection were higher in *Bdkrb1*^{-/-} group compared to the WT. However, the myeloperoxidase activity showed no difference between the infected groups. Furthermore, it was measured the levels of cytokines in BAL and *Bdkrb1*^{-/-} showed lower levels of IL-1B and IL-10 compared to WT group. In conclusion, *Bdkrb1*^{-/-} mice exhibited increased neutrophil infiltration, correlating with reduced bacterial recovery in BAL, lung, and liver.

Keywords: Lung Infection; *Pseudomonas aeruginosa*; Murine model.

3435 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

EFFECTS OF SHORT-CHAIN FATTY ACIDS (SCFA) ON RESPIRATORY SYNCYTIAL VIRUS-INDUCED NECROPTOSIS IN MURINE MACROPHAGES

CASTRO, M.S.¹; SANTOS, L.D.¹; CHAVES, A.N.¹; BORGES, S.G.¹; ANTUNES, K.H.¹; PORTO, B.N.²; DE SOUZA, A.P.D.¹. 1. PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL, PORTO ALEGRE - RS - BRASIL; 2. UNIVERSITY OF MANITOBA, MANITOBA - CANADA.

Respiratory syncytial virus (RSV) is the leading cause of respiratory infections in childhood, causing acute viral bronchiolitis. The disease causes airway inflammation and edema, and the secretion of pro-inflammatory cytokines can contribute to airway damage. Studies show that RSV can induce programmed cell death in macrophages through the regulation of TNF-mediated signaling pathways. Necroptosis is a regulated process induced by the activation of RIPK1, RIPK3, and MLKL proteins. When necroptosis is inhibited, RSV disease in animals is reduced. Short-chain fatty acids (SCFAs), metabolites of the gut microbiota, are emerging as a therapeutic alternative for RSV infection, showing a reduction in viral load after treatment. Therefore, this study aimed to analyze the effects of SCFAs on RSV-induced necroptosis in murine peritoneal macrophages. Peritoneal macrophages were obtained by the lavage of the peritoneal cavity of BALB/c mice 4 days after intraperitoneal injection of 4% thioglycollate. Macrophages were plated in 24-well plates at a concentration of 3x10⁵ cells/300µL and incubated for 24h. After this period, adherent cells were treated for 24h with acetate, butyrate, and propionate (500µM). After this period, the cells were stimulated with RSV 3x10⁵ for 6h. PCR analyses for RIPK3 and MLKL were performed. Data was analyzed using GraphPad 10. Treatment with SCFA significantly decreased mRNA expression of RIPK3 (p<0.01 for butyrate and propionate; p<0.0001 for acetate) and MLKL (p<0.05 for butyrate and propionate; p<0.01 for acetate). The data found in this study provide information of an important role of SCFA in the necroptosis pathways during RSV infection.

Keywords: Respiratory Syncytial Virus;Short Chain Fatty Acids;Necroptosis.

3439 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Lipid droplet participation in the *Listeria monocytogenes* pathogenesis

GIL, H.G.¹; SOUZA, T.D.S.²; FERREIRA, M.F.²; DA CRUZ, G.I.P.¹; SILVA, A.R.²; BOZZA, P.T.²; PEREIRA-DUTRA, F.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Listeria monocytogenes is a ubiquitous Gram-positive bacterium responsible for listeriosis, a severe foodborne illness with high hospitalization in vulnerable populations. This intracellular pathogen employs sophisticated strategies to invade and replicate within diverse host cells, including hepatocytes, which serve as a primary site of multiplication and a reservoir for systemic dissemination. Lipid droplets (LDs), once considered inert lipid storage sites, are now recognized as dynamic cellular organelles integral to both host immune responses and pathogen survival. Previous investigations have demonstrated a critical role for LDs in *L. monocytogenes*'s immune evasion during macrophage infection. However, these organelles' precise function in other crucial cell types, such as hepatocytes, remains largely undefined. To address this knowledge gap, an investigation into lipid metabolism and LD accumulation in *L. monocytogenes*-infected hepatocytes was initiated using a standardized HUH-7 cell line model. Preliminary gene expression analysis of lipid metabolism markers revealed a general reduction in the relative expression of most genes examined, suggesting a broad repression of lipid metabolism in infected hepatocytes. Intriguingly, however, a notable exception was observed for the Fatty Acid-Binding Protein 4 (FABP4) gene, which exhibited a distinct trend toward increased expression. This differential regulation points to a complex and specific metabolic reprogramming within the host cell during infection. These findings suggest that *L. monocytogenes* may selectively manipulate host lipid metabolism, potentially by upregulating FABP4 to facilitate the acquisition of essential fatty acids for its growth or to modulate host immune responses. Understanding the intricate interplay between *L. monocytogenes* and host lipid metabolism in hepatocytes holds significant implications for identifying novel host-directed therapeutic targets to combat this formidable pathogen.

Keywords: *Listeria monocytogenes*;Lipid Droplet;Immunometabolism.

3450 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Hyperimmune bovine colostrum as a source of anti-SARS-CoV-2 neutralizing antibodies

GASPAR, E.B.¹; BASTOS, A.P.²; ORTS, D.J.B.³; DA COSTA, H.H.M.³; HONORIO, N.N.⁴; PRUDENCIO, C.R.⁵; SILVA, L.P.⁶; BONATTO, C.⁶; ADRIANI, P.P.⁷; SOARES, G.O.⁸; DOMINGUES, R.¹; DE GASPARI, E.N.⁵; PORTILHO, A.I.⁹; MARTINS, M.F.¹; MACHADO, M.A.¹; BRANDÃO, H.D.M.¹; DIAVAO, J.¹; CAMPOS, M.M.¹; CARVALHO, W.A.¹. 1. EMBRAPA, JUIZ DE FORA - MG - BRASIL; 2. EMBRAPA, CONCÓRDIA - SC - BRASIL; 3. FEDERAL UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. FEDERAL UNIVERSITY OF JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 5. ADOLFO LUTZ INSTITUTE, SÃO PAULO - SP - BRASIL; 6. EMBRAPA, BRASÍLIA - DF - BRASIL; 7. SÃO PAULO UNIVERSITY, SÃO PAULO - SP - BRASIL; 8. FEDERAL UNIVERSITY OF VIÇOSA, JUIZ DE FORA - MG - BRASIL; 9. ADOLFO LUTZ INTITUTE, SÃO PAULO - SP - BRASIL.

Introduction: The global need for affordable and scalable immunoprophylaxis strategies against SARS-CoV-2 has renewed interest in bovine colostrum as a source of neutralizing antibodies. This study aimed to evaluate the immunogenicity and neutralizing capacity of colostrum from cows immunized with SARS-CoV-2 recombinant receptor-binding domain (RBD) proteins.

Methods: Pregnant cows received three intramuscular immunizations at 45, 30, and 15 days before calving with recombinant SARS-CoV-2 RBD produced in HEK293F cells and formulated with either Quil-A saponin or aluminum hydroxide (Alum) as adjuvants. Antibody responses in serum and colostrum were analyzed by indirect ELISA for total IgG, IgG1, IgG2, and avidity index. Neutralizing activity was assessed by competitive ELISA. Statistical analyses were performed using two-way ANOVA and Tukey's test ($P < 0.05$).

Results: Both adjuvanted formulations induced robust systemic and mucosal humoral responses. Vaccinated animals showed significantly higher anti-RBD IgG titers in serum and colostrum compared to controls ($P < 0.001$; Mann-Whitney test). Quil-A induced higher serum IgG1 and IgG2 levels than Alum ($P < 0.01$). The competitive ELISA confirmed neutralizing activity of colostrum antibodies, with inhibition of RBD-ACE2 binding exceeding 80% in the Alum group and 96% in the QuilA group.

Conclusions: Immunization of pregnant cows with recombinant RBD and adjuvants efficiently induced RBD-specific antibodies in colostrum with demonstrated neutralizing capacity. These findings support the use of hyperimmune bovine colostrum as a scalable, safe and cost-effective source of passive immune protection.

Keywords: Bovine colostrum ;Covid-19;neutralizing activity.

3452 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Investigating the impact of skin colonization by *Staphylococcus* spp. on *Leishmania amazonensis* infection and inflammasome activation in a murine model

LOPES, C.D.A.; DE MELO, D.K.C.; LIMA-JÚNIOR, D.D.S.; ZAMBONI, D.S.. UNIVERSITY OF SÃO PAULO (USP), RIBEIRÃO PRETO - SP - BRASIL.

Cutaneous leishmaniasis (CL), caused by *Leishmania* parasites, is characterized by chronic skin lesions whose severity varies depending on multiple factors, including host-microbe interactions. Growing evidence indicates that the skin microbiota plays a key role in modulating disease progression. In murine models, germ-free mice infected with *Leishmania major* develop smaller lesions than conventional mice, and recolonization with *Staphylococcus epidermidis* restores lesion development. Similarly, co-infection with *S. aureus* exacerbates skin pathology and increases the production of pro-inflammatory cytokines such as IL-1 β and IL-17. In clinical settings, CL lesions from patients infected with *L. braziliensis* have shown a predominance of *Staphylococcus* spp., which correlates with elevated expression of IL-1 α/β , suggesting skin microbiota regulates inflammasome activation. To investigate the contribution of different skin-colonizing *Staphylococcus* species to *Leishmania amazonensis* infection, we developed a murine model of sequential colonization followed by parasitic challenge. C57BL/6J mice were topically colonized on the ear five times at 4-day intervals with isolates from human skin: *S. aureus* (AD04.E17), *S. epidermidis* (NIHLM087), or *S. xylosus* (42C08). Colonization was confirmed via ear swabs cultured on Mannitol Salt Agar. Subsequently, mice were infected intradermally in the ear with 10^6 *L. amazonensis*-PH8. A control group received parasite infection only. We are currently optimizing the colonization protocol and monitoring lesion development across groups. This model aims to elucidate whether and how different *Staphylococcus* species modulate the inflammatory response and inflammasome activation during *Leishmania* infection.

Keywords: Staphylococcus sp;Leishmania amazonensis;inflammasome.

3455 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Intranasal treatment with OM-85 during respiratory syncytial virus infection and its effects on the innate immune response

POPPE, J.G.¹; DE SOUZA, A.P.D.¹; ANTUNES, K.H.¹; BORGES, S.G.¹; ZANATTA, A.¹; FRIES, F.¹; DUTRA, S.¹; PASQUALI, C.²; STEIN, R.T.¹. 1. PUCRS, PORTO ALEGRE - RS - BRASIL; 2. OM-PHARMA, MEYRIN - SUICA.

Acute viral bronchiolitis caused by **respiratory syncytial virus** (RSV) is one of the main respiratory diseases in children under two years old, characterized by intense lower airway inflammation. RSV accounts for a high number of hospitalizations and infant deaths, with a greater impact in resource-limited countries such as Brazil. Despite advances such as vaccines and monoclonal antibodies (e.g., Palivizumab), therapeutic options remain limited due to high cost and restricted access, reinforcing the need for new strategies to reduce disease severity.

Broncho-Vaxom® (OM-85), an oral bacterial extract composed of lysates from 21 pathogenic respiratory strains, is widely used to prevent recurrent respiratory infections. Its action involves dendritic cell activation, type I interferon induction, and modulation of inflammatory responses. Previous studies show that prophylactic intranasal OM-85 can protect against viral infections, including RSV.

To investigate the mechanisms of this protection, we conducted experiments in **nude mice** (BALB/c Foxn1^{-/-}), deficient in T lymphocytes but with preserved innate immunity. Animals received intranasal OM-85 for five consecutive days before RSV infection, plus a booster six hours later. Five days post-infection, bronchoalveolar lavage (BALF) and lung tissues were collected for analysis.

Pretreatment with OM-85 reduced viral load and increased alveolar macrophages and regulatory dendritic cells, confirming protection mediated by innate immunity. In immunocompetent BALB/c mice, OM-85 also modulated immune cell populations, notably reducing **ST2⁺** cells (consistent with ILC2s), suggesting a role in regulating these cells in the antiviral response to RSV.

Keywords: innate immunity modulation; type 2 innate lymphoid cells (ILC2s); respiratory viral infection model.

3463 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

O-GlcNAcylation as an Integrator of Metabolic State and Immune Response against *Leishmania major*

DINIZ, L.R.V.; NAVEGA, A.V.; DE OLIVEIRA, J.L.; DIAS, W.B.; FREIRE-DE-LIMA, C.G.; TODESCHINI, A.R.. IBCCF/UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Leishmania major infection triggers a localized immune response, with macrophages playing a dual role: they can eliminate the parasite via nitric oxide (NO) and pro-inflammatory cytokines like interferon gamma, or favor its persistence depending on activation state. The M2 macrophage profile, linked to reduced NO and increased glycolysis, shows elevated UDP-GlcNAc and O-GlcNAcylation, a post translational modification regulated by OGT (adds GlcNAc) and OGA (removes it). Though involved in macrophage polarization in cancer and viral infections, its role in leishmaniasis under metabolic conditions like type 1 diabetes is unclear.

To investigate further, this study evaluated how modulating O-GlcNAcylation affects the immune response to *L. major* infection under normo and hyperglycemic conditions. C57BL/6 mice, which show a resistant Th1 response, and BALB/c mice, susceptible with a Th2 profile, were used. In vitro, bone marrow derived macrophages from BALB/c mice were infected with *L. major* promastigotes (MOI 1:10), cultured in Schneider's Drosophila medium, and treated with 10 µM TMG (OGA inhibitor) or OSMI-1 (OGT inhibitor), before or after infection. Parasite burden was measured between days 7 and 10. In vivo, hyperglycemia was induced in C57BL/6 mice by streptozotocin (50 mg/kg, 5 days), followed by infection and TMG treatment; BALB/c mice received OSMI-1 to delay paw swelling.

In vitro, OSMI-1 reduced parasite burden and promastigote release and growth, suggesting macrophage polarization toward a resistant phenotype and direct interference with parasite metabolism. In vivo, OSMI-1 reduced paw swelling in BALB/c mice, while TMG impaired resistance in C57BL/6, accelerating edema and worsening infection, especially in hyperglycemic animals.

These findings indicate that O-GlcNAcylation balance is a crucial regulatory axis in macrophage–*Leishmania* interactions and a promising therapeutic target, especially for patients with metabolic comorbidities, like type 1 diabetes.

Keywords: Macrophage polarization; *Leishmania*.

3465 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

NLRP3 Inflammasome is a Critical Driver of Hepatic Granulomatous Immunity in *Leishmania infantum* Infection

DE OLIVEIRA, I.M.¹; CHAVES, M.M.²; MADEIRA, J.C.C.¹; BARBOSA, A.L.¹; DE ALMEIDA, L.¹; CORRADI, P.S.³; ISHIMOTO, A.¹; BATAH, S.S.¹; XAVIER JUNIOR, J.T.¹; HERRERA, A.J.R.¹; BECERRA, A.D.M.¹; DE SÁ, K.⁴; TAKIYA, C.M.²; MENEZES, G.³; FABRO, A.T.¹; CRUZ, A.K.¹; ZAMBONI, D.S.¹. 1. USP, RIBEIRÃO PRETO - SP - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. UFMG, BELO HORIZONTE - MG - BRASIL; 4. YALE, NEW HAVEN - ESTADOS UNIDOS DA AMÉRICA.

Visceral leishmaniasis (VL) is caused by *Leishmania* species such as *L. infantum* and *L. donovani* and caused around 5482 deaths in 2021 deaths (GBD, 2025). A better understanding of the disease pathology and host resistance mechanisms, is critical for development of immunomodulatory drugs and vaccines for this disease. In this work, we showed that patients with symptomatic visceral leishmaniasis have higher serum levels of Caspase-1, IL-18 and IL-1b when compared with non-infected patients, suggesting a role of the inflammasome in the disease development. To further study the impact of inflammasome activation in visceral leishmaniasis, we established a mouse model of VL using a strain of *Leishmania infantum* that trigger visceral disease when injected intraperitoneally. Initially, we searched for strains with a high potential of inducing visceral disease in mice and identified a strain that effectively induces visceral in C57BL/6 mice via intraperitoneal infection. Using this model of *L. infantum* infection, we demonstrated that the NLRP3 inflammasome is activated in liver of infected mice and contributes for host resistance. We showed that NLRP3 deficient mice had higher parasite loads when compared with control mice. Although NLRP3 deficient animals showed higher parasite levels, their livers were less inflamed and had impaired granuloma formation, suggesting a dual role of NLRP3 inflammasomes in controlling parasite replication and inducing inflammation. Interestingly, in infected patients higher levels of IL-1b and IL-18 directed correlated with higher levels of pro-inflammatory cytokines. Taken together, this work establishes a relevant mouse model of *L. infantum* visceral disease and reveals the NLRP3 inflammasome as a critical component for disease resistance.

Keywords: Leishmaniasis; Innate Immunity; Tropical neglect disease.

3468 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Selective effect of S1P/S1PR1 pathway modulation on in vitro viral infection of human tonsils: a comparative study of SARS-CoV-2 and Influenza A

TORO, D.M.¹; CAPELINI, C.E.²; DE SOUZA, M.V.O.¹; DA SILVA, M.H.P.¹; DA CRUZ, E.D.D.S.F.³; LEME, V.D.C.²; MARTINS, R.B.⁴; SORGI, C.A.⁵; TAMASHIRO, E.²; ANSELMO-LIMA, W.T.²; VALERA, F.C.P.²; NETO, E.D.A.¹. 1. DEPARTAMENTO DE BIOLOGIA CELULAR E MOLECULAR E BIOAGENTES PATOGENICOS, FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - FMRP, UNIVERSIDADE DE SÃO PAULO-USP, RIBEIRÃO PRETO - SP - BRASIL; 2. DEPARTAMENTO DE OFTALMOLOGIA, OTORRINOLARINGOLOGIA E CIRURGIA DA CABEÇA E PESCOÇO, FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - FMRP, UNIVERSIDADE DE SÃO PAULO-USP, RIBEIRÃO PRETO - SP - BRASIL; 3. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - FMRP, UNIVERSIDADE DE SÃO PAULO-USP, RIBEIRÃO PRETO - SP - BRASIL; 4. FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO (FCFRP/USP), RIBEIRÃO PRETO - SP - BRASIL; 5. DEPARTAMENTO DE QUÍMICA, FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO - FFCLRP, UNIVERSIDADE DE SÃO PAULO - USP, RIBEIRÃO PRETO - SP - BRASIL.

Palatine tonsils and adenoids act as key sites of immune response to respiratory pathogens, including SARS-CoV-2 and Influenza A virus (H1N1). This study aimed to investigate the role of the sphingosine-1-phosphate (S1P) signaling pathway, with a focus on the S1PR1 receptor, in viral replication and the regulation of the immunometabolic response in human tonsillar cells infected in vitro. For this, primary cultures of dissociated human tonsillar cells were pre-treated for 24 hours with W146 (a selective S1PR1 antagonist) or SKI-II (an inhibitor of S1P synthesis), followed by infection with SARS-CoV-2 or H1N1 (MOI = 2) for 48 hours. Viral load and mRNA levels of S1PR1, PGC1A (a transcriptional coactivator associated with mitochondrial biogenesis), and IFNA (type I interferon) were quantified by RT-qPCR. SARS-CoV-2 infection led to reduced expression of S1PR1 compared to H1N1, suggesting suppression of S1P/S1PR1 signaling. PGC1A expression was slightly lower in the SARS-CoV-2 group than in the H1N1 group, indicating a trend toward decreased mitochondrial activation ($p = 0.0286$). IFNA expression was similar between groups, with a slightly higher mean in SARS-CoV-2. Blocking S1PR1 with W146 and inhibiting S1P synthesis with SKI-II significantly reduced SARS-CoV-2 viral load, but had no impact on H1N1. W146 treatment also reduced PGC1A and IFNA expression, suggesting a functional relationship between S1P/S1PR1 signaling, mitochondrial metabolism, and type I interferon response. These findings highlight the relevance of the S1P/S1PR1 axis in SARS-CoV-2 infection, but not in H1N1, in human tonsillar tissue and support its potential as a selective therapeutic target.

Keywords: Immunometabolism; SARS-CoV-2; Influenza A virus (H1N1).

3470 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Zileuton, 5-lipoxygenase inhibitor, as a new promising treatment to severe malaria

SANTOS, L.L.D.O.; MELO, N.; SANTANA, L.F.D.; SILVA, L.P.; RICCI, M.F.; MACHADO, F.S.. UFMG, BELO HORIZONTE - MG - BRASIL.

Malaria affects many people worldwide. The inflammatory host's response that occurs during the pathogenesis drives the outcome of the disease. In this context, it's important to investigate new targets that regulate immune response. Zileuton is a 5-lipoxygenase inhibitor used for the prophylaxis and treatment of chronic asthma. *Plasmodium berghei* ANKA (PbA) recapitulates in many aspects the human severe malaria, including cerebral malaria. C57BL/6 mice were infected (IF) or not with 10^5 erythrocytes parasitized with PbA and subjected to different treatment regimens. Zileuton (Zi; 60mg/kg) administration was initiated at 3^o (Z3) or 5^o (Z5) day post infection (dpi) and continued for 10 days. Clinical parameters were evaluated daily and at 6dpi, the animals were submitted to brain intravital microscopy and histopathological analysis. Our results demonstrate that despite the similar parasitemia, Z3 and Z5 treatments increased the animals survival when compared to the untreated IF mice that showed precocious death associated with hemorrhage lesions, higher pyknotic nucleus cells, and perivascular cellularity in the brain when compared to both treatments. Z5 treatment increased the number of adhered and bearing cells in the brain when compared to untreated IF mice. In the liver, untreated IF mice showed more edema and hemorrhage areas when compared to Z5. In the spleen, untreated IF presents higher edema and lymphoid tissue disorganization when compared to Z3 and Z5 treated IF mice. In the lung, Z5 demonstrated parenchymal protection when compared to untreated IF, which shows loss in the tissue architecture. The colon of untreated IF mice presents more damage associated with inflammation, edema, degeneration and hemorrhage areas when compared to the Z5 treated mice. Collectively, our results demonstrate that Zileuton has a tissue protective effects and may be a new potential strategy to the severe malaria infection. Financial support: CAPES, Fapemig, CNPq. Keywords: Zileuton; *Plasmodium* ; Cerebral malaria.

3473 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Hemopressin and Arachidonoyl 2'-Chloroethylamide prevent Chikungunya virus-induced change of CB1 receptor subcellular localization in human skeletal muscle

SILVA, G.D.S.¹; FRAGA JUNIOR, V.D.S.¹; DA SILVA, J.O.¹; BORGES, A.C.N.²; COSTA, A.G.B.¹; BENJAMIM, C.F.¹. 1. FEDERAL UNIVERSITY OF RIO DE JANEIRO - UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. FEDERAL UNIVERSITY OF THE STATE OF RIO DE JANEIRO - UNIRIO, RIO DE JANEIRO - RJ - BRASIL.

Chikungunya virus (CHIKV) is an alphavirus transmitted by mosquitoes of the genus *Aedes*, specifically *Aedes aegypti* and *Aedes albopictus*. CHIKV infection is responsible for the development of Chikungunya disease (CHIK). In 2025, Brazil reported 113.975 cases and 177 deaths attributed to CHIK. Main symptoms include high fever ($\geq 38^\circ\text{C}$), arthralgia, myalgia, maculopapular rash and encephalitis. Despite the approval of the VLA1553 vaccine (Butantan-Valneva) in Brazil in 2025, initial vaccine rollout will target high-risk areas and vulnerable groups. Thus, alternative treatments remain necessary, especially for 40% of cases progressing to chronic disease with lasting inflammation, muscle and joint pain. Therefore, cannabinoids have emerged as potential myogenic and anti-inflammatory as demonstrated in model of SINDBIS. Thus, we investigated the effects of the Arachidonoyl 2'-chloroethylamide (ACEA), a CB1 receptor agonist, and the Hemopressin (HP), a CB1 receptor antagonist and inverse agonist, on immortalized human skeletal muscle cells (CL48) infected with CHIKV. Our analyses determined that a multiplicity of infection (MOI) of 0.1 was optimal, maintaining similar cell viability across conditions for 24 hours. Cells were pretreated or not with ACEA (1.0 μM) or HP (10.0 μM) for 1 hour prior to infection. A shift in the subcellular localization of CB1 receptors was observed upon CHIKV infection, which was partially prevented by cannabinoids treatment. Furthermore, CHIKV infection impaired the differentiation of myoblasts into myotubes, whereas cannabinoid compounds showed potential as supportive agents in promoting myogenic differentiation. Moreover, pretreatment with HP significantly inhibited the secretion of inflammatory mediators IL-6, IL-8 and IL-23 ($P < 0,05$). In summary, our findings suggest that cannabinoids might confer protective effects in the context of CHIKV infection in skeletal muscle.

Keywords: Cannabinoid compounds; Chikungunya virus; Skeletal muscle cells .

3483 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Expression of MIC-A/B and HLA-E ligands in tissue fibroblasts under inflammatory stimuli in localized cutaneous leishmaniasis caused by *Leishmania braziliensis*

SILVA JUNIOR, J.L.¹; DE MOURA, R.G.¹; COVRE, L.P.¹; JABOR, P.N.¹; FALQUETO, A.¹; AKBAR, A.²; GOMES, D.C.D.O.¹. 1. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 2. UNIVERSITY COLLEGE LONDON, LONDON - INGLATERRA.

Localized cutaneous leishmaniasis (LCL), caused by *Leishmania braziliensis*, is an infectious disease of major relevance in Brazil, characterized by an intense inflammatory and cytotoxic response that contributes to its immunopathogenesis. Our group has previously highlighted the role of senescent T cells in the pathology of this disease. Senescent fibroblasts, traditionally considered structural cells, also play an active role in the immune response through the senescence-associated secretory phenotype (SASP). As a consequence, SASP leads to the expression of stress ligands in surrounding tissue cells, enhancing immunopathology and local inflammation. This study aimed to investigate the expression of pro-inflammatory cytokines (IL-1 β , IL-6, IL-8, IL-15, and IL-18), the senescence marker p16, and the NK cell ligands MIC-A/B and HLA-E in tissue fibroblasts. To this end, lesion biopsies from LCL patients and healthy individuals were analyzed, and the expression of these markers was assessed by immunofluorescence. The results revealed increased expression of pro-inflammatory cytokines in the lesions. Furthermore, LCL was associated with the accumulation of p16⁺ fibroblasts and elevated expression of MIC-A/B and HLA-E ligands. Collectively, the data highlight the influence of the inflammatory microenvironment on the modulation of NK cell ligands in fibroblasts, which may contribute to the chronicity and severity of the disease. These findings broaden our understanding of the immunopathological mechanisms underlying LCL and suggest new potential therapeutic targets.

Keywords: Inflammation; Cellular senescence; Localized cutaneous leishmaniasis.

3484 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

***Cryptococcus neoformans* is modulated by cytokines and respond in an immune profile-dependent manner**

DA SILVA JUNIOR, E.B.¹; DE AMORIM, L.H.¹; CARDOSO, M.F.¹; LIANI, S.A.C.¹; VALDEZ, A.F.¹; CRUZ, L.F.¹; COVRE, L.P.²; MORROT, A.³; DE LIMA, L.F.¹; GUEDES, H.L.D.M.³; NIMRICHTER, L.¹; DECOTE-RICARDO, D.⁴; FREIRE-DE-LIMA, C.G.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 3. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 4. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, SEROPÉDICA - RJ - BRASIL.

Cryptococcus neoformans (CN) is the primary etiological agent of cryptococcosis, responsible for 200,000 deaths annually. CN is remarkable for its pathogenicity across a wide range of species, including mammals, moths and amoebae. The CN-phagocytes interaction reveal striking similarities, suggesting a conserved intracellular pathogenic strategy. Our group recently demonstrated that *Cryptococcus* genus has a diverse pathogenic strategy, tending to subvert host immune responses through morphological and molecular alterations. Furthermore, the ability of CN to dynamically alter its morphology and repertoire of virulence factors in response to microenvironmental changes highlights its remarkable phenotypic plasticity. The hypothesis of this work is that **CN is highly proficient at sensing changes and signaling molecules within the inflammatory microenvironment, such as cytokines, and uses its phenotypic plasticity to enhance its survival within the host.** For this, we cultured 10⁶ yeasts of CN H99 with 10ng of recombinant cytokines IL-4 and IFN- γ during 48h. Our results indicate that rIL-4-treated yeasts exhibit larger diameters and thicker capsules, whereas rIFN- γ -treated yeasts are smaller and have a thinner capsule. Furthermore, rIFN- γ exposure promotes a higher frequency of budding, while rIL-4 exerts more modest effects. rIL-4-treated yeasts also reduce MHCII and CD86 expression on the surface of murine macrophages, a phenomenon not observed in macrophages infected with rIFN- γ -treated yeasts. In addition, *Galleria mellonella* larvae are more susceptible to infection by rIL-4-treated yeasts but are partially resistant to infection by rIFN- γ -treated yeasts. Finally, both rIL-4- and rIFN- γ -treated yeasts decrease nitrite production by murine macrophages and can evade phagocytosis in a contact-independent manner. This work introduces a novel approach to understanding cryptococcal pathogenesis by dissecting the impact of cytokine-mediated modulation on CN yeast cells.

Keywords: Cryptococcosis; Cytokines; Immunomodulation.

3489 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Analysis of the bystander cytotoxicity potential in cells with a senescent phenotype in distinct forms of cutaneous leishmaniasis

BARBOZA, B.D.A.¹; LOPES, P.O.¹; DE MOURA, R.G.¹; DE CARVALHO, L.P.²; DE CARVALHO FILHO, E.M.³; BORGES, V.³; COVRE, L.P.¹; AKBAR, A.⁴; GOMES, D.C.D.O.⁵. 1. NÚCLEO DE DOENÇAS INFECCIOSAS, UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 2. INSTITUTO GONÇALO MONIZ - FUNDAÇÃO OSWALDO CRUZ,, SALVADOR - BA - BRASIL; 3. INSTITUTO GONÇALO MONIZ - FUNDAÇÃO OSWALDO CRUZ, SALVADOR - BA - BRASIL; 4. 3. DIVISÃO DE MEDICINA, UNIVERSITY COLLEGE LONDRES, LONDRES - REINO UNIDO; 5. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITORIA - ES - BRASIL.

Localized cutaneous leishmaniasis (LCL) has been associated with cellular senescence, a cell state frequently arising from DNA damage. Studies have demonstrated that T lymphocytes within lesional tissue of LCL exhibit elevated expression of senescence markers, such as p16, when compared to those from healthy individuals. Furthermore, these cells may acquire novel functionalities, including the capacity to mediate bystander cytotoxicity, a TCR-independent cell killing process characterized by the expression of natural killer cell receptors (NKR) like NKG2D and NKG2C on T cells. Our current findings indicate that this more inflammatory manifestation of leishmaniasis is characterized by this distinct cellular phenotype. Nevertheless, a critical objective remains to ascertain whether this phenomenon is exclusively linked to this specific disease presentation or if it can also be observed in less inflammatory conditions, such as diffuse cutaneous leishmaniasis (DCL) lesions. To address this question, the present study utilized multiparametric immunofluorescence assays to evaluate the expression of markers associated with bystander cytotoxic activity by both CD4+ and CD8+ cells, comparing LCL and DCL lesions. Our phenotypic analyses revealed that, within LCL lesional tissue, both CD4+ and CD8+ T cell populations exhibited an increased frequency of p16 and GZMB expression. While the co-expression of these lymphocyte subsets with NKRs demonstrated no statistical significance for NKG2D and NKG2C in either population. Notably, the combined phenotypic populations of CD4+NKG2D+GZMB+p16+ and CD8+NKG2D+GZMB+p16+ were not only increased in LCL lesions but also appeared to be uniquely associated with this disease. Collectively, these results reinforce the sustained cytotoxicity associated with senescence-like cells observed in LCL ulcers and suggest that this distinct process is more strongly correlated with inflammatory environmental diseases.

Keywords: American tegumentary leishmaniasis; Cellular senescence; Bystander cytotoxicity.

3505 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Influence of dysregulated metabolic environment in the function and inflammatory response of THP-1 cells infected with M. tuberculosis

GALDINO, G.; ABBIATI, Y.; LAMOGLIA, A.; BONECINI, G.; ROLLA, V.C.; DE LIMA, E.H.R.. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Patients with type 2 diabetes (T2D) have an increased risk of Mycobacterium tuberculosis (Mtb) infection, disease progression, and greater severity of tuberculosis (TB). In contrast, individuals with elevated cholesterol levels show a lower risk of active TB, along with reduced severity and mortality. Metabolic disorders impact immune responses, and evidence indicates these conditions alter defense mechanisms required for TB control. However, how dysregulated metabolic environments contributes to modulate Mtb infection remains unclear. This study investigates how high glucose and high oxLDL microenvironments shape the metabolic programming and activation of THP-1 cells infected with Mtb. THP-1-derived macrophages were stimulated with high glucose, high oxLDL, or both, simulating hyperglycemic and dyslipidemic environments, followed by infection with the H37Rv Mtb strain for 24h, 48h, 5 and 7 days. Macrophages stimulated with high oxLDL, as well as with both high glucose and oxLDL, showed inhibited intracellular mycobacterial growth at days 5 and 7 post-infection (p.i.), as determined by CFU. qPCR analysis revealed downregulation of inflammatory genes, such as IL-1 β and CCR7, in macrophages stimulated for 24h with both high glucose and high oxLDL. Furthermore, Luminex analysis of supernatants from macrophages exposed to high glucose, oxLDL, or both revealed altered profiles of cytokine and protein secretion, including changes in TNF family members: APRIL/TNFSF13 showed reduced secretion under combined high glucose and high oxLDL stimulation, whereas BAFF/TNFSF13B and sTNF-R1 were decreased with high oxLDL alone, all at 48h p.i. These findings show modulation of the high glucose and high oxLDL microenvironment in control of intracellular Mtb growth and THP-1 cells inflammatory response during TB infection

Keywords: immunometabolism; Mycobacterium tuberculosis infection; inflammatory response.

3513 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

GAS6 – Axl/MerTK signaling promotes protection against severe experimental tuberculosis

CORREA, A.A.D.S.¹; DENADAI, M.B.¹; SILVA, G.C.¹; DE ANDRADE, A.C.M.M.²; LEITE, N.P.D.S.³; MATTEUCCI, K.C.³; BONATO, V.L.D.¹; COSTA, D.L.¹. 1. USP, RIBEIRÃO PRETO - SP - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL; 3. FIOCRUZ, RIBEIRÃO PRETO - SP - BRASIL.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is the leading cause of death by an infectious disease. Modulating host immune responses has emerged as a promising strategy to improve TB control. Axl and MerTK receptors recognize GAS6-bound phosphatidylserine on apoptotic cells, triggering efferocytosis and immunomodulatory signaling pathways. We observed that Mtb infection of C57BL/6 mice results in increased Axl and MerTK expression in lung interstitial macrophages as well as enhanced GAS6 and soluble Axl and MerTK levels, suggesting that a competition by the ligand between soluble and membrane-bound receptors might occur in the infected lungs. To address this, Mtb-infected C57BL/6 mice were treated or not with GAS6 intranasally from days 14 to 28 post-infection. GAS6 treatment resulted in reduced lung bacterial loads, lower iNOS expression in alveolar macrophages, as well as reduced T-bet and FoxP3 expression in CD4⁺ T cells. Axl or MerTK deletion did not lead to differences in survival, bacterial load or lung myeloid cell profiles in response to in vivo Mtb infection alone, but experiments to test if the effects of GAS6 treatment on experimental TB are Axl and/or MerTK dependent are ongoing. In vitro, C57BL/6, Axl^{-/-} and MerTK^{-/-} bone marrow-derived macrophages (BMDMs) directly infected with Mtb and treated or not with GAS6 presented similar bacterial loads. However, when cells were indirectly infected with Mtb-containing BMDMs, to mimic efferocytosis of infected cells, bacterial loads were lower in C57BL/6 compared to Axl^{-/-} and MerTK^{-/-} cells. Importantly, GAS6 opsonization of infected BMDMs prior to challenge resulted in reduction of bacterial loads in all groups, suggesting a redundant role for Axl and MerTK in this effect. These results demonstrate that GAS6–Axl/MerTK signaling supports host protection against Mtb and identify intranasal GAS6 as a potential host-directed immunotherapy for TB. **Financial support:** FAPESP

Keywords: Mycobacteria tuberculosis; GAS6; Receptors TAM.

3515 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Combination Therapy with Anti-PD-1 or Anti-CTLA-4 and Amphotericin B Enhances Immune Response and Fungal Control in murine Paracoccidioidomycosis

LOURES, F.V.; PREITE, N.W.; DOS SANTOS, B.V.; FRANCO, F.N.. UNIFESP, SÃO JOSÉ DOS CAMPOS - SP - BRASIL.

Paracoccidioidomycosis (PCM), caused by *Paracoccidioides* spp., is a systemic fungal infection endemic to the Americas, with the highest incidence in Brazil. Antifungal treatments, including amphotericin B (AmB), itraconazole, and fluconazole, are often associated with treatment abandonment and/or long-term sequelae due to toxicity, compromising patient outcomes. Immunomodulatory therapies, such as monoclonal antibodies (mAbs) targeting checkpoint molecules CTLA-4 and PD-1—both linked to immune exhaustion through the deactivation of effector mechanisms—have shown promise in reducing fungal burden and improving survival in murine PCM and other infectious diseases. Here, we the efficacy of combined therapy using mAbs (anti-CTLA-4 or anti-PD-1) with AmB in *Paracoccidioides brasiliensis*-infected C57BL/6 mice. After six weeks of infection, mice received mAbs, AmB, or a combination of both for two weeks. Disease progression was assessed through colony-forming unit (CFU) counts, histopathology, survival analysis, and immune profiling by flow cytometry. Results demonstrated that combining anti-PD-1 or anti-CTLA-4 with AmB significantly enhanced disease control, reducing fungal load in the lungs, liver, and spleen, minimizing pulmonary damage, and increasing survival by over 60% compared to monotherapy with either anti-PD-1, anti-CTLA-4, or AmB. Additionally, anti-CTLA-4 + AmB combination therapy reduced the infiltration of dendritic cells, neutrophils, and Th1/Th17 lymphocytes into the lungs, while increasing the frequency of Tc1 cells. In contrast, anti-PD-1 + AmB therapy promoted dendritic cell and macrophage recruitment, along with an increased frequency of CD8⁺ and Th1/Th17 cells in the lungs. These findings indicate that immune modulation with mAbs synergizes with AmB's antifungal activity, providing superior disease control compared to monotherapy. Combined therapy thus represents a promising strategy for PCM, improving efficacy while mitigating long-term sequelae.

Keywords: Immunotherapy; anti-CTLA-4/anti-PD-1; Paracoccidioidomycosis.

3517 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

ABC transporters gene expression are upregulated in lesions of cutaneous leishmaniasis compared to health skin

DE SANTANA, M.B.R.¹; DE SANTANA, M.B.R.²; DE CARVALHO, L.P.². 1. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. FIOCRUZ BAHIA, SALVADOR - BA - BRASIL.

Introduction. *Leishmania braziliensis* is the main causative agent of cutaneous leishmaniasis (CL) in Brazil. Therapeutic failure has been associated with high production of inflammatory cytokines, as TNF and IL-1b, and high parasite burden. Treatment efficacy to pentavalent antimonial (SbV) varies range from 40% to 70% depending on the stage of the disease. Drug resistance in CL has been poorly addressed. The most extensively studied mechanisms involve drug efflux mediated by transmembrane proteins, primarily belonging to the ATP-binding cassette (ABC). **Aim.** To determine whether infection with *Leishmania braziliensis* results in increased expression of ABC transporter proteins.

Methods. Using our publicly available microarray data from Novais et al. (2015) and RNA-Seq data from Amorim et al. (2020), we analyzed the gene expression profiles of ABC transporters in healthy skin from healthy subjects (HS) and in CL lesion. Among the differentially transcribed genes, there were those significantly upregulated in CL lesions compared to HS and those that were downregulated. Since the increased expression of these transporters is associated with multidrug resistance, we focused on the upregulated genes. Genes encoding ABC transporters that were identified in both studies were selected, and the comparison between CL and HS was performed. Expression data for transporters present in only one study were not analyzed. **Results.** Gene expression analyses in both studies showed overexpression of ABCA1, ABCC1, ABCD1, ABCF3, and ABCG4 in CL patient lesions compared to HS. Also, our findings consistently demonstrated that *L. braziliensis in vitro* infection significantly reduces cytoplasmic accumulation of fluorescent dye in macrophages, an effect that is reversible upon pharmacological inhibition of MDR1 and MRP transporters. **Conclusion.** These results provides evidence for understanding the molecular mechanisms underlying resistance to SbV, the primary treatment for CL.

Keywords: leishmania;abc transporters;drug restistance.

3520 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

EVALUATING OF miRNA-193b AND TREM-1 AS PROGNOSTIC BIOMARKERS IN CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA BRAZILIENSIS

MARINHO, A.I.¹; ARAUJO, S.N.D.O.²; BASTOS, R.P.D.S.¹; DO AMARAL, R.V.¹; RIBEIRO, B.V.²; BENICIO, I.²; CRISTAL, J.R.S.²; PEDRO, R.B.D.S.³; AMARAL, E.H.B.³; LESSA, A.C.M.³; DE JESUS, T.S.³; CARVALHO, E.M.²; DE CARVALHO, L.P.¹; OLIVEIRA, P.R.S.³; TAVARES, N.M.². 1. UFBA/FIOCRUZ, SALVADOR - BA - BRASIL; 2. FIOCRUZ/IGM, SALVADOR - BA - BRASIL; 3. UFBA, SALVADOR - BA - BRASIL.

Leishmaniasis, a neglected tropical disease caused by protozoa of the *Leishmania* genus, affects approximately 12 million people worldwide and remains a major public health challenge. Cutaneous leishmaniasis (CL) is its most prevalent form, characterized by ulcerative skin lesions that may evolve into chronic or treatment-resistant disease. MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression with recognized roles in infectious diseases, yet their potential as biomarkers or therapeutic targets in CL remains underexplored. Previous transcriptomic analyses from our group indicated correlations between miR-193b and TREM-1 in patients with early cure (<60 days), miR-193b is associated with its target gene, CD40, demonstrating the important role of this miRNA in the expression of genes related to the inflammatory response observed in LCL. However, this correlation was not observed in patients with delayed healing (>60 days). To further investigate, we quantified the expression of miR-193b and TREM-1 by RT-qPCR in biopsy samples from *L. braziliensis* infected patients in two endemic regions of Brazil (Corte de Pedra and Jiquiriçá, Bahia). Patients were stratified according to healing time and compared with healthy controls. While miR-193b expression showed no significant differences in the patient biopsies compared controls. However, the expression of TREM-1 was significantly upregulated in the biopsies of patients compared to healthy controls. No significant changes were observed between cured and treatment failure groups, for miR-193b and TREM-1. Altogether, our findings suggest that TREM-1, and possibly miR-193b, are associated with faster lesion healing and favorable outcomes in CL. These results provide novel insights into host-pathogen interactions and point to potential biomarkers for guiding personalized therapeutic strategies.

Keywords: miR-193b;TREM-1;cutaneous leishmaniasis.

3522 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Pre-exposure to Lipopolysaccharide Promotes Pneumonia Development by Low-Virulence *Streptococcus pneumoniae* Serotype 19F in Balb/c Mice: Effects on Survival, Hematological Profile, and Bacterial Dissemination

MAGALHÃES, V.D.S.¹; GASPAR, S.L.D.F.¹; QUINTERO PAMPLONA, G.¹; LIMA, K.C.N.¹; PEREIRA, R.C.D.S.¹; DIAS, A.G.D.S.²; CARVALHO, H.C.D.O.F.¹; DE CARVALHO, M.E.F.¹; LISBOA, L.F.T.¹; PAMPLONA, T.C.D.D.L.¹; ABUCATER, B.L.D.S.M.¹; MONTEIRO, M.C.¹. 1. UNIVERSIDADE FEDERAL DO PARÁ, BELÉM - PA - BRASIL; 2. UNIVERSIDADE FEDERAL PARÁ, BELÉM - PA - BRASIL.

Streptococcus pneumoniae pneumonia remains a major cause of morbidity and mortality worldwide. Low-virulence serotypes, such as 19F, have difficulty establishing infection in animal models, which limits the study of their pathophysiology. Lipopolysaccharide (LPS), a component of Gram-negative bacteria, acts as an immune modulator and may alter susceptibility to secondary infections. **Objective:** In this context, we investigated whether pre-exposure to LPS favors the establishment of pneumonia induced by *S. pneumoniae* 19F in Balb/c mice. **Methodology:** Twenty-five animals were divided into five groups (n=5/group). Three groups received intraperitoneal LPS (0.1 µg/mL) and, after five days, were challenged intranasally with 5×10^6 (LSP1), 5×10^7 (LSP2), or 10^8 (LSP3) CFU/mL. The SP group was infected only with 10^8 CFU/mL of *S. pneumoniae* (without LPS), while the Sham group received distilled water via IP and Müller-Hinton broth via IN. The animals were monitored for seven days, assessing survival, hematological profile, and bacterial load in the lung, bronchoalveolar lavage fluid (BALF), and nasopharyngeal lavage fluid (LNF). **Results:** No significant differences in survival were observed among SP, LSP1, LSP2, and LSP3. In the leukogram, only LSP3 showed leukopenia ($7.6 \times 10^3/\mu\text{L}$) and neutropenia (19.2%) compared with the SP group ($17.7 \times 10^3/\mu\text{L}$; 32.2%). The SP group showed no detectable bacterial growth, whereas in LSP3 animals, dissemination was observed in the lung, BALF, and LNF. **Conclusion:** Pre-exposure to LPS was decisive in potentiating disease severity, favoring hematological alterations and promoting broader bacterial dissemination. These findings highlight the role of LPS as a modulator of innate immunity, facilitating the progression of pneumococcal infection in a controlled experimental model.

Keywords: *Streptococcus pneumoniae*; Lipopolysaccharide; Pneumonia model.

2693 - Category: IMMUNOPHARMACOLOGY (IP)

Assessing the immunomodulatory effect of an aminoadamantane derivative against the inflammatory response mediated by Sars-Cov-2

CLARINDO, F.A.¹; FERREIRA, G.M.¹; MARQUES, D.P.D.A.¹; RIBEIRO, Á.L.¹; DE CARVALHO, L.D.²; M. FILHO, O.A.³; DA FONSECA, F.G.¹; SABINO, A.D.P.⁴. 1. LABORATÓRIO DE VIROLOGIA BÁSICA E APLICADA (LVBA), DEPARTAMENTO DE MICROBIOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS, UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. DEPARTAMENTO DE BIOLOGIA E BIOTECNOLOGIA DE MICRORGANISMOS, UNIVERSIDADE ESTADUAL DE SANTA CRUZ (UESC), ILHÉUS - BA - BRASIL; 3. GRUPO INTEGRADO DE PESQUISAS EM BIOMARCADORES, RENE RACHOU INSTITUTE, OSWALDO CRUZ FOUNDATION, BELO HORIZONTE - MG - BRASIL; 4. LABORATÓRIO DE HEMATOLOGIA CLÍNICA, EXPERIMENTAL E MOLECULAR, DEPARTAMENTO DE ANÁLISES CLÍNICAS E TOXICOLÓGICAS, FACULDADE DE FARMÁCIA, UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

During COVID-19 pandemic, many infected patients experienced severe systemic inflammation, associated with a cytokine storm caused by an imbalanced immune cells recruitment and activation. In this context, immunomodulatory interventions could be effective as they attenuate the patient's abnormal viral-mediated inflammatory response. Modifications of currently available molecules are desirable as they can potentially improve their affinity and pharmacology at a lower cost than the current treatments for COVID-19. In this regard, 3F4, a synthetic compound derived from the Amantadine molecule and with in vitro antiviral effect against SARS-CoV-2, was evaluated in the context of the coronavirus-specific inflammatory response. Samples from healthy donors were employed for the study of soluble mediators (N=9) by Luminex magnetic bead assay after short-term culture with inactivated virus (iSARS-CoV-2) in the presence of 3F4. Control cultures without iSARS-CoV-2 were also evaluated in the absence and presence of 3F4. The aminoadamantane derivative presented a robust modulatory activity on the soluble mediators, reducing the levels of chemokines and proinflammatory cytokines, such as CCL3, CCL4, IL-1b, IL-6, IFN-g and IL-17, following the short-term stimulation. Furthermore, reduced levels of regulatory cytokines (IL-4, IL-5), and growth factors (FGF-basic, IL-2) were also observed following 3F4 treatment. Aiming to obtain an in-depth assessment of the immunomodulatory effects of 3F4 at a cellular level, immunophenotyping assays are currently being performed to evaluate lymphocytes, monocytes and granulocytes regarding their activation following short-term culture with iSARS-CoV-2. However, there is still the need to understand the drug's mechanisms for its anti-inflammatory effect and proceed with the in vivo tests. Nevertheless, these preliminary results associated with the robust antiviral effects of 3F4, indicate its potential as a therapeutic strategy for COVID-19.

Keywords: COVID-19; Immunomodulatory activity; Aminoadamantane derivative.

2714 - Category: IMMUNOPHARMACOLOGY (IP)

Evaluation of the immunomodulatory and antiviral activity of the Amazonian extracts BretMUNE® and BretVIR® against Influenza A virus infection

TEIXEIRA, C.W.¹; REIS, E.V.D.S.¹; GUAMÁN-BURNEO, M.C.²; FERREIRA, G.M.¹; RIBEIRO, Á.L.¹; DA FONSECA, F.G.¹; DOS REIS, J.G.A.C.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. LABORATORIOS NOVANDINA DEL ECUADOR S.A., QUITO - ECUADOR.

The influenza virus (H1N1) has been the cause of the 2009 flu pandemic, which affected over 214 countries and territories with over 18000 deaths. Since then, seasonal circulation of other H1N1 strains has been identified worldwide, being one of the primary causes of acute respiratory infections. In addition to the pandemic potential of H1N1, the high mutation rate of the virus compromises the effectiveness of specific antiviral treatments against viral infection. Moreover, currently available treatments are costly, limiting access to these therapies, indicating the necessity to explore new, effective, and affordable therapeutic strategies. In that sense, this study aimed to evaluate two Amazonian extracts, BretMUNE® and BretVIR®, in MDCK.2 and human PBMCs as potential treatments for H1N1. Both extracts have already been evaluated and have shown promising results against other viruses such as SARS-CoV-2, which motivated their selection for this study.

The results for the antiviral assays revealed low cytotoxicity for both extracts in cell culture, with viability remaining above 80% for MDCK.2 cells. Additionally, viral titration indicated that both BretVIR® and BretMUNE®, have the potential to reduce viral load compared to untreated MDCK.2 cells. As for the assessment of the immunomodulatory potential of both extracts, PBMCs isolated from healthy donors were isolated and incubated with H1N1 virus before treatment with BretVIR® or BretMUNE®. After incubation, cell supernatant was collected and Luminex® assay was carried out.

Our results have shown that both extracts promote a modulation of the immune response, with a decrease in excessive pro-inflammatory cytokines and an increase in IFN- γ production, suggesting efficient antiviral activation without inducing hyperinflammation. Therefore, new tests are underway to clarify the mechanisms of immune modulation and viral inhibition, and their potential as anti-influenza antiviral candidates.

Keywords: Influenza A; Amazonian extracts; Virus.

2737 - Category: IMMUNOPHARMACOLOGY (IP)

Liposomes from Cnidoscolus urens extract negatively modulates neutrophil oxidative metabolism

DE GASPERI, R.M.¹; LIMA, V.D.S.¹; HASSELSTROM, G.H.¹; ALVES, N.P.¹; EMERICK, G.L.¹; FERRARINI, S.R.¹; MACHADO, C.M.M.²; SINHORIN, V.D.G.³; ALBIERO, L.R.¹. 1. UFMT, SINOP - MT - BRASIL; 2. USP, RIBEIRÃO PRETO - SP - BRASIL; 3. UFMT, CUIABÁ - MT - BRASIL.

Introduction: *Cnidoscolus urens* (*C. urens*; tiredness) has been used in folk medicine for its anti-inflammatory applications. In some inflammatory pathologies, there is intense recruitment of neutrophils to the inflamed microenvironment, activating them, culminating in the massive release of the toxic content of their granules and oxidizing species, capable of damaging the tissue, causing an injury process. Therefore, there is an attractive approach for modulating neutrophil effector functions using natural compounds present in *C. urens*. Objective: In vitro evaluation of the anti-inflammatory effects of *C. urens* leaf extract incorporated into liposomes on the oxidative metabolism of human neutrophils. Methodology: Multilamellar liposomes containing the extract were developed from phosphatidylcholine and cholesterol. The cytotoxicity test of the plant under study was performed using the MTT method (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide). The oxidative metabolism of neutrophils stimulated with zymozam was evaluated by measuring the production of superoxide anion ($O_2^{\cdot-}$), produced by neutrophils, using the NBT method (nitroblue tetrazolium) (CEP/UFMT/Sinop Opinion: 7.430.150). Results: Neutrophils from healthy individuals stimulated with Zimozam and when treated with *C. urens* produced less formazam compared to the positive control, that is, the extract in the form of liposomes showed inhibition in the production of superoxide anion by approximately 34%. Conclusion: Liposomes containing *C. urens* negatively modulate the oxidative metabolism of neutrophils, since superoxide anion is a potent free radical with microbicidal effect, which also exacerbates inflammation in patients where neutrophils are involved. Thus, superoxide anion may contribute as a potent therapeutic effect for inflammatory diseases such as autoimmune diseases, where neutrophils play an important role.

Keywords: Neutrophils; Liposomes; *Cnidoscolus urens*.

2751 - Category: IMMUNOPHARMACOLOGY (IP)

Anti-IL7R α Antibody Slows Progression of Childhood Lymphoblastic Leukemia in vivo

FEITOZA, J.M.S.¹; DE LIMA, F.M.D.S.¹; FERREIRA, A.P.S.¹; FONSECA, J.R.F.¹; CAVALCANTE, M.D.S.¹; DE AZEVEDO, A.C.²; YUNES, J.A.¹; RODRIGUES, G.O.L.³; DURUM, S.K.³; SALLES, P.P.Z.¹. 1. BOLDRINI RESEARCH CENTER, CAMPINAS - SP - BRASIL; 2. DR. DOMINGOS A. BOLDRINI CHILDREN'S CENTER FOR HEMATOLOGIC RESEARCH, CAMPINAS - SP - BRASIL; 3. NATIONAL INSTITUTES OF HEALTH, BETHESDA - ESTADOS UNIDOS DA AMERICA.

Lymphoblastic leukemias are hematologic neoplasms of B and T cells and represent the leading cause of cancer-related death in children. The α subunit of the interleukin-7 receptor (IL7R α) is essential for lymphocyte development and maturation and is present in its wild-type isoform in acute lymphoblastic leukemia (ALL). Mutant variants of IL7R α account for 2–10% of pediatric B- and T-ALL cases. Despite cure rates exceeding 80%, around 30% of children with ALL relapse, highlighting the need for more effective therapies. Here, we evaluated the therapeutic potential of a chimeric antibody in B-ALL subtypes. Experiments were approved by animal (#0036-2022) and human research ethics (#59137022.9.0000.5376) committees. NSG or NOD-SCID mice were intravenously xenotransplanted with 3–5 $\times$ 10⁶ cells from 05 B-ALL subtypes (ETV6-RUNX1, High-Hyperdiploid, B-other with and without IL7R mutations, and TCF3-PBX1). Animals (n = 6/group) were treated weekly with 250 μ g of therapeutic antibody or isotype control (IgG1) intraperitoneally for ~6 weeks, starting when $\geq$ 0.5% hCD45⁺ cells were detected in the blood of mice. Treatment efficacy was monitored weekly by flow cytometry (FlowJo X), along with body weight as part of clinical assessment. Mice were euthanized upon reaching $\geq$ 50% hCD45⁺ cells or showing clinical signs. Body weight was expressed relative to baseline (mean $\pm$ SD). Kaplan–Meier curves were analyzed with the log-rank test (GraphPad Prism). The antibody reduced leukemic progression in all tested samples, with a significant survival benefit in the High-Hyperdiploid subtype ($p=0.048$). Final analysis confirmed leukemic infiltration in bone marrow, liver, and spleen in all samples. These results support the *in vivo* efficacy of the anti-IL7R α antibody and its potential as a therapeutic strategy in ALL. Future studies will assess organ infiltration at defined endpoints and explore protocols with dexamethasone combined or not with the antibody.

Keywords: Therapeutic Antibody;Immunotherapy;Target therapy.

2837 - Category: IMMUNOPHARMACOLOGY (IP)

Assessment of the anti-inflammatory activity and phytochemical composition of the ethanolic extract of *Persea americana* Mill. (avocado)

APRIGIO, F.; ALVES, R.F.; MARTINS, B.D.P.; DE PONTES, P.H.P.C.; REIS, P.A.; COELHO, M.G.P.. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

The use of plants in traditional medicine is an ancient practice, and new therapeutic agents can be derived from the extraction of secondary metabolites. This study investigated the safety and efficacy of *Persea americana* Mill. (avocado), focusing on the seed of the fruit. The main objective was to evaluate the anti-inflammatory potential of the ethanolic extract of *P. americana* seed (EEPa) in both *in vitro* and *in vivo* models. The EEPa was obtained by macerating the seeds in 96% ethanol for 15 days. Phytochemical analyses revealed a rich and complex composition present in the EEPa. Thin-layer chromatography (TLC) detected the presence of terpenoids and flavonoids. Additionally, high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) indicated the presence of phenolic compounds and flavonoids, including catechin, epicatechin, orientin, rutin, vitexin/isovitexin, and coumarin. *In vitro* assays demonstrated that EEPa exhibited no significant cytotoxicity in RAW 264.7 cells. Moreover, it was able to inhibit nitric oxide (NO) production in LPS-stimulated macrophages, with a more pronounced effect observed at concentrations of 50 to 100 μ g/mL. *In vivo*, EEPa also demonstrated anti-inflammatory activity in the thioglycollate-induced peritonitis model, reducing leukocyte migration into the peritoneal cavity at all tested doses. Differential analysis of the exudate confirmed decreased migration of mononuclear cells to the site of inflammation, as well as a reduction in total protein concentration and the levels of the chemokines MCP-1/CCL2 and KC/CXCL-1. In conclusion, the results suggest that EEPa contains bioactive compounds with promising potential for the development of new therapies against inflammation. This study highlights the therapeutic potential of medicinal plants, emphasizing the importance of phytochemical and pharmacological research for developing safe and effective therapeutic approaches in modern medicine.

Keywords: *Persea americana*;Inflammation;Phytochemistry.

2891 - Category: IMMUNOPHARMACOLOGY (IP)

LQB-462 and LQB-464 in an *in vitro* inflammation model: a study of mechanism of action

FIGUEIREDO, L.M.¹; JUSTO, G.¹; THIMÓTEO, R.R.C.¹; DIAS, A.G.¹; COSTA, D.D.²; BRITO, D.C.³. 1. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO DE PESQUISAS BIOMÉDICAS - HNMD - MARINHA DO BRASIL, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Inflammation is an essential physiological response to infection or tissue damage and can progress to chronic conditions (Abbas; Lichtman; Pillai, 2015). Although anti-inflammatory drugs are widely used, many are associated with significant adverse effects. Thus, the development of new compounds aims to preserve therapeutic efficacy while reducing toxicity. (Yu et al., 2018; Thimóteo et al., 2021). Cyclic analogs of the terpenyl nitron LQB-278—specifically LQB-462 and LQB-464—obtained by hybridizing the terpene geraniol with the nitron PBN at the Organic Chemistry Laboratory, Institute of Chemistry, UERJ, previously demonstrated *in vitro* anti-inflammatory activity in the Griess assay. These highly purified compounds displayed IC₅₀ values of 12.83 µM (LQB-462) and 13.82 µM (LQB-464).

The overall objective of this study was to investigate the mechanisms underlying the anti-inflammatory activity of LQB-462 and LQB-464 in an *in vitro* model using LPS-activated (1 µg/mL) RAW 264.7 macrophages. Specific aims included: assessing cell migration using Wound Healing and Boyden chamber assays; quantifying IL-1β and IL-10 cytokines secretion by ELISA; and analyzing the expression of CD14 and CD44 markers by flow cytometry.

Results showed that both cyclic analogs inhibited cell migration; however, only the LQB-464 derivative significantly inhibited IL-1β production. IL-10 levels remained elevated following treatment with both derivatives, though below those induced by LPS stimulation alone. This preliminary study indicates that both derivatives exhibit promising anti-inflammatory activity, warranting further investigation to elucidate their mechanisms of action *in vitro* and *in vivo*.

Keywords: Mechanisms the anti-inflammatory activity; Inflammation; LQB.

2900 - Category: IMMUNOPHARMACOLOGY (IP)

Unveiling the immunomodulatory potential of Amazon Stingless bee Propolis

CAVALCANTE, T.T.A.; FARIA, J.A.Q.A.; SANTOS, F.G.P.; DE OLIVEIRA, A.L.L.; SILVA, J.L.S.; DO NASCIMENTO, A.H.; PIMENTEL, I.H.M.. UNIVERSIDADE FEDERAL DO AMAPÁ - UFAM, MANAUS - AM - BRASIL.

The antioxidant and immunomodulatory properties of *Apis mellifera* propolis have been well investigated; however, the characterization and activity of stingless bee propolis have received little attention in the literature. This study aimed to characterize the immunomodulatory profile of *Duckeola ghilianii* propolis extract (DGE), a stingless bee from the Amazon rainforest. The maximal non-cytotoxic concentration of the DGE, defined previously by our group, was used to treat PMA-differentiated human THP-1 macrophages for 24 h. The intracellular reactive oxygen species levels were assessed using the probe 2',7'-dichlorofluorescein diacetate. The cytokine profile was quantified using BD Cytometric Bead Array (CBA) Human Th1/Th2/Th17 cytokine Kit in the supernatant of the DGE-pretreated and subsequently stimulated macrophages with lipopolysaccharide (LPS). Our results showed that DGE reduced the ROS levels in basal condition and, also in response to H₂O₂-induced oxidative stress. Analysis of the cytokine profile revealed that while LPS stimulation alone is known to increase pro-inflammatory cytokines, DGE significantly mitigated the LPS-induced increase in IL-6, TNF-α, and IL-1β. Furthermore, analysis of the supernatant from DGE-pretreated cells revealed a significant reduction in IL-10 levels and an increase in CXCL-8 levels. In summary, these findings indicate that DGE exhibits anti-inflammatory properties through the reduction of ROS and pro-inflammatory cytokine levels. Additionally, an immunomodulatory effect was observed that deviates from classical anti-inflammatory modulation, characterized by a decrease in IL-10 levels and the elevation of CXCL8/IL8 levels, which warrants further investigation. Further studies related to the immunomodulatory potential of propolis extracts are crucial for advancing scientific knowledge and, also for empowering meliponiculture.

Keywords: Propolis extract; immunomodulation; cytokines.

2949 - Category: IMMUNOPHARMACOLOGY (IP)

Humanization of an Anti-IL7R α Antibody by CDR-grafting for Pediatric Leukemia Therapy

FONSECA, J.R.F.¹; DE LIMA, F.M.D.S.¹; FEITOZA, J.M.S.¹; FERREIRA, A.P.S.¹; BRIGIDO, M.D.M.²; MARANHÃO, A.Q.²; SALLES, P.P.Z.³. 1. UNICAMP, CAMPINAS - SP - BRASIL; 2. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL; 3. UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS - SP - BRASIL.

Monoclonal antibodies (mAbs) are a significant advance in immunotherapy for diseases including cancer. Our group developed murine anti-IL7R (CD127) mAbs that recognize both wild-type and mutated forms of the protein linked to pediatric acute lymphoblastic leukemia (ALL). These antibodies showed potential by reducing disease progression. However, murine mAbs can trigger a Human Anti-Mouse Antibody (HAMA) response, compromising treatment safety and efficacy. To address this, we applied CDR-grafting, transferring complementarity-determining regions (CDRs) onto human antibody frameworks to reduce immunogenicity. This study aimed to develop and characterize a humanized version of the murine anti-CD127 antibody. The most homologous human VH and VL sequences were selected via IgBlast to receive the CDRs. Backmutations were introduced in key regions to preserve affinity. The humanized sequences were cloned into AbVec vectors with constant human regions and expressed in ExpiCHO-S cells. Structural integrity was confirmed by SDS-PAGE. Affinity for IL7R was measured by surface plasmon resonance (SPR), showing a KD of 0.29 nM. Specificity was evaluated by labeling CD127-positive and negative cells. A competition assay by flow cytometry confirmed that murine and humanized mAbs bind the same epitope. Preclinical tests in NSG mice engrafted with RS4;11 cells showed the humanized antibody reduced leukemic cell levels in blood and organs. This study demonstrated the successful humanization of an anti-CD127 antibody, maintaining high affinity and specificity, and showing promising efficacy in reducing leukemic cells in preclinical models. This validates its potential as a safer and more effective therapy for pediatric leukemia.

Keywords: CDR-grafting;Antibody;Leukemia.

2953 - Category: IMMUNOPHARMACOLOGY (IP)

Genetic and pharmacological manipulation of hybridomas

DA SILVA, A.F.A.¹; MATAVEL, A.C.D.S.². 1. PONTIFÍCIA UNIVERSIDADE CATÓLICA DE MINAS GERAIS, BETIM - MG - BRASIL; 2. FUNDAÇÃO EZEQUIEL DIAS, BELO HORIZONTE - MG - BRASIL.

Hybridomas are cells obtained by fusing B lymphocytes with myeloma cells, combining the ability to produce specific antibodies with the immortality of tumor lines. They are widely used in the large-scale production of monoclonal antibodies (mAbs), which have significant applications in diagnostics, research, and therapy. Given their economic relevance, strategies to enhance or regulate antibody production are of great interest. In this study, we aimed to evaluate the effects of both pharmacological treatments and genetic manipulation on antibody expression in hybridomas. Cells were cultured in RPMI medium with 10% fetal bovine serum and subjected either to treatment with selected drugs or transfection with specific plasmid clones. Antibody levels were measured by indirect ELISA seven days after induction. Initially, calcium and potassium channel blockers and high extracellular potassium concentrations did not significantly affect antibody expression. However, a follow-up experiment revealed that two of the tested drugs were capable of reducing antibody production. In contrast, genetic manipulation induced a discrete but significant increase in expression, particularly with clone 2, which elevated antibody levels by approximately 25%. These findings suggest that genetic approaches are promising tools to enhance antibody production, while some pharmacological agents may also play a role in regulating hybridoma output. Further investigation into these mechanisms may contribute to technological advances in monoclonal antibody development and bioproduction.

Keywords: Hybridomas, Monoclonal antibodies, ;Genetic manipulation, Pharmacological modulation,;ELISA.

2973 - Category: IMMUNOPHARMACOLOGY (IP)

Humanized anti-CD127 antibodies for acute lymphoblastic leukemia therapy: In vitro assaysDE LIMA, F.M.D.S.; FONSECA, J.R.F.; FEITOZA, J.M.S.; FERREIRA, A.P.S.; SALLES, P.P.Z.. UNICAMP, CAMPINAS - SP - BRASIL.

Approximately 10% of pediatric cases of Acute Lymphoblastic Leukemia (ALL) harbor oncogenic mutations in the interleukin-7 receptor alpha chain (IL7R α). Our group previously developed murine anti-IL7R α monoclonal antibodies capable of recognizing both wild-type and mutant forms of the receptor, demonstrating therapeutic efficacy in vitro and in vivo. However, clinical application requires antibody humanization. This study aimed to evaluate, in vitro, the humanized anti-IL7R α antibodies (Hu.mAbs). Humanization of the murine antibody mAb#64 and ELISA testing were performed by OAK Bioscience. One IgG1 Hu.mAb was produced in vitro by transfecting ExpiCHO-S cells with pcDNA 3.4 TOPO plasmids containing the humanized variable region sequences. Antibody integrity was confirmed by SDS-PAGE, and binding affinity was measured using Surface Plasmon Resonance (SPR). Specificity was assessed by flow cytometry using leukemic cell lines expressing or lacking IL7R. A total of eight Hu.mAb clones and one chimeric antibody were generated. The degree of humanization exceeded 80% for both heavy and light chains. The antibodies were intact, displaying molecular weights of ~50 kDa (heavy chain) and ~25 kDa (light chain). Flow cytometry confirmed specific binding of the antibodies to IL7R-positive cells, with no staining in IL7R-negative lines, indicating high specificity. SPR analysis revealed a dissociation constant (KD) of 6.67 nM for clone 2, produced in-house, indicating strong binding affinity. However, in a competition assay performed by flow cytometry using the Ba/F3.CPT cell line expressing mutant IL7R, no competition was observed between the murine and humanized antibodies, suggesting that the humanization process may have altered the binding epitope. Although the humanized antibody retained high affinity for IL7R, the data suggest that it may recognize a different epitope. To further assess the therapeutic potential of the humanized antibody in ALL, in vivo studies are planned.

Keywords: THERAPEUTIC ANTIBODIE;HUMANIZED ANTIBODIE;IL7R ALPHA.

2988 - Category: IMMUNOPHARMACOLOGY (IP)

Therapeutic effect of melanocortin agonist (AP1189) during severe dengue virus infectionDA FONSECA, T.C.M.; MARTINS, J.R.; BATISTA, V.L.; DIAS, A.S.L.; SILVA, L.S.; SANTOS, A.L.D.C.; GOULART, P.P.G.; TEIXEIRA, M.M.; QUEIROZ JUNIOR, C.; COSTA, V.V.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Dengue is a widespread tropical disease caused by an Orthoflavivirus arbovirus and remains a major public health issue. The four dengue virus serotypes (DENV1–4) can cause both the classical form and severe dengue (SD). SD is characterized by an exacerbated inflammatory response and/or impaired or delayed resolution of inflammation. Melanocortins are endogenous peptide hormones with well-established anti-inflammatory properties, extensively studied in murine models. For example, macrophages with activated melanocortin receptors have been shown to enhance efferocytosis and phagocytosis, as well as inhibit leukocyte chemoattraction. AP1189 is a biased agonist of melanocortin receptors 1 and 3, with previously demonstrated effects in murine models of coronavirus infection. This study aimed to evaluate the therapeutic potential of AP1189 during dengue virus infection. We employed a previously established model of SD using A129 mice, which lack type I interferon receptors. Animals were divided into control, infected untreated, and infected AP1189-treated groups, evaluated at two time points: 3 and 5 days post-infection (dpi). Control mice received 30 μ L of 0.1 M PBS intraplantarly in the right hind paw pad. Infected mice received the same volume containing DENV-2 (2×10^4 PFU). Treated animals received AP1189 (50 mg/kg) in β -cyclodextrin, diluted in PBS, administered orally every 24 hours. Mice were monitored daily, and euthanasia was performed at 3 and 5 dpi. DENV inoculation induced thrombocytopenia increased clinical scores, weight loss, elevated inflammatory cytokines and chemokines, and higher viral loads in plasma and liver. AP1189 prevented thrombocytopenia and clinical score increase at both time points without impairing viral control. Indeed, AP1189 treatment reduced CCL5 plasma levels and did not affect mast cell degranulation. Overall, these results suggest that melanocortin agonists may be useful as adjuvant therapy to treat dengue infection.

Keywords: DENGUE;MELANOCORTIN;INFLAMATION.

3005 Category: IMMUNOPHARMACOLOGY (IP)

Hydroalcoholic Leaf Extract of *Xanthosoma sagittifolium* (L.) increased the Viability of Fanconi Anemia Type C Lymphoblasts in vitro

DE ANDRADE, E.A.M.; JUSTO, G.; THIMÓTEO, R.R.C.; SAKURAGUI, C.M.. UERJ, RIO DE JANEIRO - RJ - BRASIL.

Fanconi Anemia (FA) is a rare disease caused by mutations in one of 23 DNA repair genes, whose encoded proteins repair damages induced by DNA-intercalating agents. Patients present with congenital malformations and aplastic anemia, with a high predisposition to cancer development. Current treatments, still palliative, include androgenic anabolic steroids and bone marrow transplantation. This study aims to evaluate the effect of the leaf extract of *Xanthosoma sagittifolium* (common Taioba) on the *in vitro* viability of FANCC lymphoblasts, seeking active compounds that may reduce the oxidative stress characteristic of the disease and promote cell viability. Fanconi type C (FANCC) cells are known to overexpress P-glycoprotein (Pgp), which is associated with multidrug resistance. Therefore, before testing the extract's effect, the impact of the inhibitor verapamil on Pgp expression was assessed using rhodamine 123 by flow cytometry. FEPS and K562 cell lines served as positive and negative controls, respectively, confirming high Pgp expression in FEPS and absence in K562. The FANCC (NEO) cell line showed high Pgp activity, which was inhibited by verapamil, demonstrating effective Pgp inhibition without cytotoxic effects, as evaluated by Trypan Blue exclusion assay (0.02%). The effect of the crude hydroalcoholic extract from *Xanthosoma sagittifolium* leaves was tested at increasing concentrations in NEO and LIU (corrected FANCC control) cell lines through *in vitro* Trypan Blue exclusion and MTT assays. The extract showed no cytotoxicity at the tested concentrations and increased cell viability in NEO cells compared to controls at 100 µg/mL and 200 µg/mL. These results suggest that common Taioba is a potential nutraceutical food source for the therapeutic management of Fanconi Anemia.

Keywords: Fanconi type C (FANCC); P-glycoprotein (Pgp); *Xanthosoma sagittifolium*.

3015 - Category: IMMUNOPHARMACOLOGY (IP)

Anti-inflammatory potential of *Pereskia aculeata* Mill. (Ora-Pro-Nóbis) extracts in inhibiting microglial activation

OLIVEIRA, S.C.D.A.; DE PONTES, P.H.P.C.; DOS SANTOS, P.D.M.A.; COELHO, M.G.P.; REIS, P.A.. UERJ, RIO DE JANEIRO - RJ - BRASIL.

Neuroinflammation refers to inflammatory processes in the central nervous system (CNS) that lead to the activation of resident immune cells, primarily microglia, which play a crucial role in maintaining neural homeostasis. When dysregulated, this activation contributes to the development of neurodegenerative diseases such as Alzheimer's and Parkinson's, due to the excessive release of inflammatory mediators like nitric oxide (NO). This study investigated the potential of *Pereskia aculeata* Mill., commonly known as ora-pro-nóbis, to modulate the inflammatory response of activated microglia *in vitro*. Aqueous and hydroalcoholic extracts were prepared from the plant powder using different extraction methods to maximize the yield of bioactive compounds. Thin-layer chromatography (TLC) analysis of *Pereskia aculeata* revealed a more evident presence of flavonoids in the hydroalcoholic extract (EHPa) compared to the aqueous extract (EAPa). Microglial cell cultures were stimulated with lipopolysaccharide (LPS) to induce an inflammatory profile and subsequently treated with dexamethasone (positive control) or increasing doses of the extracts (10, 25, 50, 75, 100 mg). Anti-inflammatory activity was assessed by quantifying nitrite levels using the Griess reaction, while cell viability was evaluated by the MTT reduction assay. The results showed that the hydroalcoholic extract (5 g/L) was more effective in reducing NO production compared to the aqueous extract, indicating greater anti-inflammatory potential and no significant cytotoxicity. In contrast, the aqueous extract reduced cell viability at certain concentrations. These findings suggest that the phytochemical composition, which depends on the extraction method, directly influences the biological activity of the extract. Therefore, *Pereskia aculeata* may represent a promising complementary therapeutic strategy to modulate neuroinflammation and help delay neurodegenerative processes mediated by excessive microglial activation.

Keywords: Neuroimmunology; *Pereskia aculeata* Mill.; Microglia.

3017 - Category: IMMUNOPHARMACOLOGY (IP)

In Vitro Study of the Anti-Inflammatory Potential of *Pterodon polygalaeflorus* Fractions in a Neuroinflammation Model

DE PONTES, P.H.P.C.; OLIVEIRA, S.C.D.A.; THIMÓTEO, R.R.C.; GAYER, C.R.M.; COELHO, M.G.P.; REIS, P.A.. UERJ, RIO DE JANEIRO - RJ - BRASIL.

Neuroinflammation plays a pivotal role in the development and progression of several neurodegenerative diseases. Microglial activation, when exacerbated, contributes to neuronal damage through the release of pro-inflammatory mediators. This study investigates the anti-inflammatory potential of bioactive fractions isolated from *Pterodon polygalaeflorus* (sucupira) in BV2 murine microglial cells stimulated with lipopolysaccharide (LPS). After extraction and fractionation of the plant material, selected fractions were tested in vitro for their effects on cell viability and inflammatory response.

Fractions were obtained through extraction and chromatographic separation of *P. polygalaeflorus* seeds. Phytochemical analysis by thin-layer chromatography (TLC) revealed the presence of terpenoids. Nitric oxide (NO) quantification demonstrated that fraction 3 (F3) significantly reduced NO release at concentrations $\geq 20 \mu\text{g/mL}$ ($p=0.0049$ at $20 \mu\text{g/mL}$; $p<0.0001$ at $40\text{--}60 \mu\text{g/mL}$) with an estimated IC_{50} of $\sim 30 \mu\text{g/mL}$. Fraction 4 (F4) also inhibited NO production in a dose-dependent manner, reaching statistical significance only at higher concentrations ($p<0.0001$ at $40\text{--}60 \mu\text{g/mL}$; ANOVA). Cytotoxicity assessment using Annexin V-FITC/PI staining by flow cytometry has also been initiated, indicating potential protective effects of these fractions on cell viability. MTT assays also confirmed that the tested concentrations were not cytotoxic to BV2 cells. Further experiments will include immunofluorescence to characterize microglial morphology and inflammatory mediator release. The expected outcome is the identification of fractions capable of modulating the inflammatory profile of BV2 cells, indicating the presence of compounds with therapeutic potential in neuroinflammatory conditions. This study reinforces the importance of exploring natural products as innovative strategies for the modulation of microglial activation in the central nervous system.

Keywords: Microglia; *Pterodon polygalaeflorus*; Neuroinflammation.

3032 - Category: IMMUNOPHARMACOLOGY (IP)

EVALUATION OF THE EFFECT OF THERAPY WITH HYBRID IONIZABLE NANOPARTICLES ON GENE EXPRESSION AND RANKL PRODUCTION IN CO-CULTURE OF OSTEOBLASTS AND PROSTATE ADENOCARCINOMA CELLS

MAIA, B.F.F.¹; DE OLIVEIRA, M.F.C.¹; SEIXAS, E.R.¹; RODRIGUES, P.H.¹; ANTÔNIO, P.L.A.¹; ALVES, M.T.R.²; ALVES, É.A.R.¹; DA SILVA, W.N.²; GUIMARAES, P.P.G.²; CARVALHO, K.A.B.³. 1. FUNDAÇÃO OSWALDO CRUZ - INSTITUTO RENÉ RACHOU, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. FUNDAÇÃO OSWALDO CRUZ - INSTITUTO RENÉ RACHOU, BEL - MG - BRASIL.

Bone metastasis (BMT) is a serious complication of prostate cancer, present in up to 80% of advanced cases, reducing patient survival to less than 3 years. This process is driven by the overexpression of RANKL, a cytokine that stimulates the exacerbated activation of osteoclasts and accelerates bone resorption, resulting in events such as fractures, chronic pain, and hypercalcemia. In Brazil, therapies such as bisphosphonates and denosumab reduce symptoms but do not increase survival and can cause serious adverse effects. In this context, ionizable polymeric lipid nanoparticles (LNPs) emerge as a promising alternative as a nanoplatform for gene therapy. This project aims to develop LNPs loaded with siRNA for RANKL to reduce its expression in osteoblasts (SaOs-2), prostate adenocarcinoma cells (PC3-Luc2), and in co-cultures of both. LNPs were formulated by microfluidics at LabNano/UFMG, using a 10:1 (w/w) ratio between ionizable lipids (7C1 and C14PEG2K) and siRNARANKL. Two populations of LNPs with distinct siRNA sequences (siRNA-RANKL1 and siRNARANKL2) were formulated and characterized: diameters of 91.47 nm and 104.8 nm; polydispersity indices of 0.125 and 0.215; zeta potentials of -6.17 mV and -2.25 mV; and siRNA concentrations of 130 ng/ μL and 180.1 ng/ μL , respectively. To date, RANKL gene expression levels were evaluated by RT-qPCR in SaOs-2 after treatment with three concentrations of LNPs (10, 50, and 100 nM). The siRNA-RANKL1 formulation showed greater silencing efficacy at 100 nM, a concentration defined for future PC3-Luc2 and co-culture assays. In parallel, AlamarBlue cytotoxicity assays did not indicate toxicity in SaOs-2. Therefore, a significant reduction in RANKL expression is expected in the treated groups compared to the control group. As a next step, we intend to evaluate the proposed therapy in vivo in immunodeficient mouse models of prostate cancer with BMT to simulate the clinical conditions of the disease in a complete biological system.

Keywords: RANKL; Ionizable lipid-polymer nanoparticles; Gene Therapy.

3043 - Category: IMMUNOPHARMACOLOGY (IP)

CB2 agonists as immunomodulators of macrophage and dendritic cell responses to mycobacterial infection

VALERIANO, J.D.P.¹; ANDRADE-SILVA, M.²; PEREIRA-DUTRA, F.³; SEITO, L.N.⁴; BOZZA, P.T.⁵; ROSAS, E.C.⁴; DE SIQUEIRA, P.F.⁶; SILVA, M.F.D.S.C.⁶; HENRIQUES, M.D.G.M.D.O.⁷. 1. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, NITERÓI - RJ - BRASIL; 2. DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO, BRAZIL, SÃO PAULO - SP - BRASIL; 3. IMMUNOPHARMACOLOGY LABORATORY, IOC, OSWALDO CRUZ FOUNDATION, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO - RJ - BRASIL; 4. LABORATORY OF APPLIED PHARMACOLOGY, FARMANGUINHOS, OSWALDO CRUZ FOUNDATION, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO - RJ - BRASIL; 5. IMMUNOPHARMACOLOGY LABORATORY, IOC, OSWALDO CRUZ FOUNDATION, RIO DE JANEIRO, BRAZIL., RIO DE JANEIRO - RJ - BRASIL; 6. IMMUNOBIOLOGY OF INFLAMMATION LABORATORY, IMMUNOBIOLOGY DEPARTMENT, IB, UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI, NITERÓI - RJ - BRASIL; 7. PRESENT ADDRESS: LABORATORY OF CELLULAR AND MOLECULAR PHARMACOLOGY, DEPARTMENT OF CELL BIOLOGY, IBRAG, UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO - RJ - BRASIL.

Tuberculosis remains a global health challenge due to its capacity for immune evasion, latent persistence, and increasing antibiotic resistance. In the lungs, *Mycobacterium tuberculosis* infects macrophages and dendritic cells, which are key innate immune cells and initiators of immune response. This pathogen reprograms cell metabolism to survive and replicate intracellularly, contributing to persistent inflammation and disease progression. The cannabinoid receptor type 2 (CB2) is highly expressed in immune cells and has been implicated in the regulation of inflammatory responses. Host-directed therapies targeting the immune system have gained attention as promising alternatives. Here, we investigate the immunomodulatory effects of CB2 agonists on macrophages and dendritic cells in the context of *Mycobacterium bovis* BCG infection. In macrophages [(J774A.1)], the pretreatment with GP1a, JWH-133, and GW833972A significantly reduced TNF- α , IL-6, PGE₂, and nitric oxide release induced by inactivated BCG. GP1a also downregulated pro-inflammatory and lipid metabolism-related genes, suppressed lipid droplet formation, and inhibited NF- κ B, triggered by BCG stimulation. In murine bone marrow-derived dendritic cells, post-infection treatment with GP1a decreased BCG-induced TNF- α and IL-1 β without impairing the expression of MHC-I, MHC-II, or CD80, suggesting preserved antigen-presenting capacity. Additionally, GP1a reduced the intracellular bacterial load in dendritic cells. These findings provide novel evidence that CB2 agonists modulate both inflammatory and metabolic pathways in innate immune responses to mycobacteria. Together, these results demonstrate consistent immunomodulatory effects of CB2 agonists across different innate immune cell types and infection models, supporting CB2 as a promising target for host-directed immunotherapy in tuberculosis.

Keywords: cannabinoid receptor;BCG;tuberculosis.

3074 - Category: IMMUNOPHARMACOLOGY (IP)

Drug screening to mitigate cytokine-induced energetic imbalance in cardiomyocytes

RODA, V.M.D.P.¹; SILVA, L.F.S.E.²; NUNES, J.P.S.²; ANDRIEUX, P.³; GONGORA, A.³; MARTÍNEZ-CONDE, C.I.Á.²; ZONZIN, G.F.²; MADEIRA, R.P.²; KALIL, J.²; CHEVILLARD, C.³; CUNHA-NETO, E.². 1. INSTITUTO DO CORAÇÃO - INCOR, SÃO PAULO - SP - BRASIL; 2. INSITUTO DO CORAÇÃO - INCOR, SÃO PAULO - SP - BRASIL; 3. INSERM, MARSEILLE - FRANÇA.

Background: Approximately 30% of Chagas Disease (CD) patients develop chronic Chagas disease cardiomyopathy (CCC), an inflammatory myocardial disease with a worse prognosis than other cardiomyopathies. Myocardial fibrosis, inflammation, and mitochondrial dysfunction contribute to the onset of heart failure and arrhythmias. Recent findings from our group and others on CCC heart tissue and cardiomyocytes *in vitro* suggest that cytokine-(IFN- γ and TNF- α) induced mitochondrial dysfunction plays a significant role in CCC pathogenesis. Targeting cytokine-induced mitochondrial dysfunction in cardiomyocytes could be a promising therapeutic approach. **Goal:** screening FDA-approved library with 2,320 compounds on IFN- γ and TNF- α -treated AC16 human cardiomyocytes to repurpose drugs that can mitigate cytokine-induced mitochondrial dysfunction. **Methods:** Screening for mitochondrial bioenergetic dysfunction was conducted by quantifying ATP using a luciferase assay. Compounds that mitigated at least 75% of the cytokine-induced reduction in ATP production were selected for validation and titration. Subsequently, the cytotoxicity of the optimal dose was evaluated. Following this, the most effective compounds were subjected mitochondrial membrane potential assays. **Results:** In the initial run, we identified 284 compounds. After validations, titration and cytotoxicity, 11 compounds improved energetic metabolism in cardiomyocytes in the presence of cytokines. In addition, 4 compounds improved cytokine induced-MMP decrease. **Conclusions: some compounds can improve cardiomyocyte bioenergetics dysfunction induced by cytokines *in vitro*, showing promise as therapeutic agents.**

Keywords: Chagas Disease;Mitochondrial Dysfunction;Drug Repurposing.

3092 - Category: IMMUNOPHARMACOLOGY (IP)

Evaluation of the oxidative metabolism of neutrophils from patients with rheumatoid arthritis treated in vitro by *Cnidoscolus urens*

CARLOS, R.M.G.¹; KONZEN, L.S.R.¹; LIMA, V.D.S.²; DE GASPERI, R.M.³; HASSELSTROM, G.H.³; ALVES, N.P.³; EMERICK, G.L.³; MACHADO, C.M.M.⁴; ALBIERO, L.R.³. 1. FACULDADE ATENAS, SORRISO - MT - BRASIL; 2. UNIVERSIDADE FEDERAL DE MATO GROSSO, CUIABÁ - MT - BRASIL; 3. UNIVERSIDADE FEDERAL DE MATO GROSSO, SINOP - MT - BRASIL; 4. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - MT - BRASIL.

Introduction: *Cnidoscolus urens* (*C. urens*; cansanção) has been used in folk medicine for its anti-inflammatory applications. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that causes joint and systemic changes, mainly due to accumulations of neutrophils, a cell of innate immunity that releases destructive enzymes, ROS and NETs which contribute to synovial degradation. Considering this, there is an attractive approach to modulating the effector functions of neutrophils, with the use of natural compounds present in *C. urens*. **Objective:** In vitro evaluation of the anti-inflammatory effects of *C. urens* leaf extract incorporated into liposomes on the oxidative metabolism of human neutrophils. **Methodology:** The cytotoxicity test of the plant under study was carried out using the MTT method (3-(4,5-dimethyl-thiazol 2-yl)-2,5-diphenyl tetrazolium bromide) and LDH (lactate dehydrogenase activity). The oxidative metabolism of neutrophils stimulated with zimozam was assessed by measuring the production of superoxide anion (O₂⁻), produced by neutrophils, using the NBT (nitroblue tetrazolium) method (Report CEP/UFMT/Sinop: 7.430.150). **Results:** Neutrophils from RA patients stimulated with Zimozam and when treated with *C. urens* produced less formazan compared to the positive control, that is, the extract exhibited inhibition in the production of superoxide anion by approximately 50%. **Conclusion:** The *C. urens* exerts a negative modulation on neutrophils oxidative metabolism, considering that superoxide anion is a potent free radical with a microbicidal effect, which also exacerbates inflammation in patients where neutrophils are involved. Therefore, tansanion could contribute as a potent therapeutic effect for RA, in which neutrophils play an important role.

Keywords: *Cnidoscolus urens*; Neutrophils; rheumatoid arthritis.

3102 - Category: IMMUNOPHARMACOLOGY (IP)

The Resveratrol Analog AR33 Attenuates 5-Fluorouracil-Induced Intestinal Mucositis

DE OLIVEIRA, L.G.; DO NASCIMENTO, M.D.; REIS, A.C.C.; GUEDES, M.C.M.R.; ALMEIDA, A.P.G.; DE SOUZA, I.L.L.; DA SILVA, A.D.; MEINEL, R.S.; REIS, M.O.; PEREIRA, L.G.L.; SEGHEO, J.A.; PASCHOALIM, M.E.D.O.; ÁVILA, T.V.; MACEDO, G.C.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Introduction: 5-Fluorouracil (5-FU) is widely used in cancer treatment but often induces intestinal mucositis, a debilitating inflammatory condition affecting up to 90% of patients. This adverse effect is partly attributed to epithelial cell apoptosis and gut dysbiosis, compromising treatment adherence and efficacy. Resveratrol (RVT), a natural polyphenol with antioxidant, anti-inflammatory, and immunomodulatory properties, has shown promising effects. However, its poor bioavailability limits clinical use. The development of analogs with improved pharmacokinetics, such as AR33, emerges as a promising strategy. **Objective:** To evaluate the effects of AR33 on 5-FU-induced intestinal mucositis in mice. **Methods and Results:** This study was approved by the Animal Ethics Committee of the Federal University of Juiz de Fora (protocol nº 005/2024). Female C57BL/6 mice were treated with 5-FU (75 mg/kg, i.p., for three days) and concomitantly with AR33 (50 mg/kg, i.p.). After five days, clinical, hematological, histological, and microbiological parameters were assessed. AR33 attenuated weight loss, reduced morbidity scores, and prevented intestinal shortening. It also improved blood leukocyte counts. However, AR33 increased MPO activity in the ileum, suggesting enhanced neutrophil recruitment. Importantly, AR33 did not interfere with antitumor activity of 5-FU, as reflected by sustained low bone marrow cell counts. Although enterobacteria levels were not significantly elevated in 5-FU-treated mice by day five, AR33 reduced their abundance, suggesting possible microbiota modulation. Additionally, in vitro assays showed that AR33 inhibited *E. coli* growth, supporting its potential antimicrobial action. **Conclusion:** AR33 showed beneficial effects on 5-FU-induced intestinal mucositis, with evidence of immunomodulatory and antimicrobial properties. Further studies are ongoing to elucidate its mechanisms. **Funding:** FAPEMIG (RED-00067-23; RED-00096-22; APQ-02177-22, APQ-01327-24)

Keywords: Intestinal mucositis; Resveratrol; Inflammation.

3147 - Category: IMMUNOPHARMACOLOGY (IP)

The Resveratrol Analog AR23 Reduces Adhesion of Mucositis-Derived Bacteria in an In Vitro Intestinal Epithelium Model

REIS, A.C.C.; DO NASCIMENTO, M.D.; DE OLIVEIRA, L.G.; DE ALMEIDA, T.C.; MACHADO, A.B.F.; DA SILVA, A.D.; MEINEL, R.S.; MACEDO, G.C.; ÁVILA, T.V.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Introduction and objective: Bacterial adhesion to the intestinal epithelium is a key step in the onset of gastrointestinal infections and inflammation. Resveratrol, a natural polyphenol found in grapes and wine, exhibits anti-inflammatory, antioxidant, and antimicrobial properties, including the ability to interfere with microbial adhesion to host cells. However, its low in vivo bioavailability limits clinical application. To address this, structural analogs have been developed to enhance biological effects. This study aimed to evaluate the ability of the resveratrol analog AR23 to modulate bacterial adhesion to enterocytes. **Methods and results:** This study was approved by the Animal Ethics Committee of the Federal University of Juiz de Fora (protocol nº 005/2024). The cytotoxicity of AR23 was assessed in J774A.1 murine macrophages using the MTT assay; 50 µM was identified as non-cytotoxic. Caco-2 cells (1×10^5 /well) were cultured for 24 h in 24-well plates. *E. coli* (1×10^6 CFU/mL), isolated from feces of mice after mucositis induction (5-FU, i.p. for 3 days), were added and incubated for 1 h. AR23 was then added and incubated for an additional 2 h. Cells were lysed with 1% Triton X-100, and lysates plated on BHI agar for CFU counting. A parallel group containing *E. coli* alone (no Caco-2 cells) was also treated with AR23 to assess direct antibacterial effects. Treatment with AR23 significantly reduced the number of CFUs recovered from Caco-2 cells, suggesting decreased bacterial adhesion. In the bacteria-only group, CFU counts were unaffected, indicating no direct bactericidal activity under the tested conditions. **Conclusion:** AR23 reduced *E. coli* adhesion to Caco-2 cells without affecting bacterial viability, indicating its potential as a modulator of host-pathogen interaction. These findings support its use as a preventive strategy against intestinal infections and warrant further studies to clarify its mechanisms and confirm efficacy *in vivo*. FAPEMIG APQ-01327-24
Keywords: Inflamação;Mucosite intestinal;Resveratrol.

3162 - Category: IMMUNOPHARMACOLOGY (IP)

Anti-inflammatory potential of the supramolecular complex between GMR, a resveratrol analog, and HP-β-cyclodextrin

SEGHE TO, J.A.; BOLOTARI, M.; GUEDES, M.C.M.R.; DE OLIVEIRA, L.G.; ALMEIDA, A.P.G.; REIS, M.O.; PEREIRA, L.G.L.; PASCHOALIM, M.E.D.O.; PAULA, P.D.L.; FABRI, R.L.; MEINEL, R.S.; DA SILVA, A.D.; MACEDO, G.C.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Introduction: Resveratrol (RVT) is a natural polyphenol with well-documented anti-inflammatory activity. However, the poor water solubility and low bioavailability limits its clinical application. To address these limitations, our group developed the synthetic analog GMR, which has demonstrated promising biological activity, even surpassing that of RVT. Nevertheless, GMR remains a poorly soluble organic compound. In this context, complexation with cyclodextrin molecules has emerged as an effective strategy to overcome solubility barriers. Therefore, this study aimed to evaluate the solubility, cytotoxicity, and anti-inflammatory properties of GMR complexed with hydroxypropyl-β-cyclodextrin (GMR/HPβCD). **Methods and Results:** First, the complex was characterized by UV-vis spectroscopy and scanning electron microscopy (SEM). Then, its impact on cell viability was assessed in J774A.1 cells (murine macrophages) using the MTT assay. Stimulated macrophages were also used to evaluate anti-inflammatory activity through nitric oxide production (Griess method) and cytokines IL-6, TNF-α and IL-10 (ELISA). UV-vis analysis revealed increased absorption bands for the complex, indicating improved solubility. SEM images showed marked morphological changes: free GMR exhibited a spiculated surface, while the complex formed larger plate-like structures, suggesting the formation of a new supramolecular arrangement. Moreover, GMR/HPβCD showed a safer cytotoxic profile, with no significant reduction in cell viability up to 400 µM. In anti-inflammatory assays, GMR/HPβCD significantly reduced NO production, at levels comparable to free GMR, and decreased proinflammatory cytokine levels, although less efficiently. **Conclusion:** GMR complexation with HP-β-cyclodextrin improved solubility, reduced cytotoxicity, and preserved, at least partially, its anti-inflammatory effects. These results demonstrate that GMR/HPβCD is a promising candidate for treating inflammatory diseases. **Financial support:** RED00067-23; RED00096-22; APQ02177-22.
Keywords: Resveratrol; Anti-inflammatory; Analog.

3203 - Category: IMMUNOPHARMACOLOGY (IP)

AR33, a resveratrol analogue, modulates inflammatory responses in zymosan-induced arthritis in mice

PASCHOALIM, M.E.D.O.; ZIMMERMANN-FRANCO, D.C.; DOS REIS, L.B.; MEINEL, R.S.; DA SILVA, A.D.; MACEDO, G.C.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Introduction and objective: Arthritis is a chronic inflammatory disease characterized by immune cell infiltration, increased production of pro-inflammatory cytokines and joint degradation. In this context, resveratrol (RVT), a polyphenol present in grapes and its derivatives, has shown promise due to its anti-inflammatory properties, including the inhibition of cytokines such as IL-1 β , IL-6, and TNF- α , frequently associated with the pathogenesis of arthritis. However, its low bioavailability limits its clinical use. To overcome this limitation, our group developed AR33, a resveratrol analogue that has demonstrated potent anti-inflammatory activity *in vitro*. Therefore, this study aimed to evaluate the effects of AR33 in a murine model of zymosan-induced arthritis. **Methods and results:** C57BL/6 mice (CEUA: 031/2016 and 045/2016) were subjected to the arthritis model by intra-articular injection of zymosan. The animals were then treated with RVT or AR33 (50mg/kg) intraperitoneally for 5 days. After the last treatment, the animals were euthanized, and joint tissues were collected for morphological analysis (HE staining), quantifying the area of inflammatory infiltrate, as well as cell number and density. The production of cytokines and the chemokine CCL-2 were assessed by ELISA. The results demonstrated that the AR33 analogue significantly reduced the infiltrate, the number of inflammatory cells, and the cell density in the joint tissue. Furthermore, it was able to inhibit the production of IL-1 β , IL-6, TNF- α , IL-12, and CCL-2 in a manner comparable or superior to RVT. Notably, only AR33 induced a significant increase in IL-10 production. **Conclusion:** The results demonstrate that the AR33 analogue exerts modulatory effects on zymosan-induced arthritis, supporting its potential as part of new therapeutic approaches for the treatment of arthritis. Additional studies are underway to elucidate its mechanisms of action. **Financial support:** FAPEMIG (RED-0067/23, RED-0096-22).

Keywords: Arthritis;Resveratrol;Inflammation.

3251 - Category: IMMUNOPHARMACOLOGY (IP)

Discovery of PQM-208: A New Synthetic Hydrazone-Triazole Prototype with Multi-Target Pharmacological Profile Against SARS-CoV-2.

REBELATO, B.¹; GONTIJO, V.S.¹; DOS REIS, J.G.A.C.²; REIS, E.V.D.S.²; MORAES, T.D.F.S.²; CLARINDO, F.A.²; FERNANDES, P.D.³; FRANÇA, P.R.D.C.³; GUEDES, I.A.⁴; DARDENNE, L.E.⁴; VIEGAS JUNIOR, C.¹. 1. UNIVERSIDADE FEDERAL DE ALFENAS, ALFENAS - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, BELO HORIZONTE - MG - BRASIL; 4. LABORATORIO NACIONAL DE CIÊNCIA COMPUTACIONAL, BELO HORIZONTE - MG - BRASIL.

Overall, the results indicate that the hydrazone-triazole derivative PQM-208 is a promising compound for the development of new multifunctional antivirals against SARS-CoV-2. Its hybrid structure, containing triazole and hydrazone units, was obtained through a rationally designed synthetic route, combining click chemistry reactions (CuAAC) and condensation with aromatic hydrazides. PQM-208 demonstrated excellent *in vitro* antiviral activity, with an IC₅₀ value of 0.573 nM—significantly more potent than reference antivirals such as remdesivir (IC₅₀ = 11.41 μ M) and nirmatrelvir (IC₅₀ = 8.08 μ M). Furthermore, it showed low cytotoxicity in Vero CCL-81 and HEK293 cells, with a CC₅₀ above 250 μ M, resulting in a selectivity index greater than 432,000. *In vivo* experimental assays of lung inflammation induced by inactivated SARS-CoV-2 are currently underway, with preliminary results indicating relevant anti-inflammatory effects, such as reduced levels of TNF- α and IFN- γ . Altogether, these findings reinforce the multitarget pharmacological profile of PQM-208 and highlight its value as a prototype for the development of innovative and effective therapies against COVID-19.

Keywords: hydrazone-triazole hybrids;Antiviral activity;SARS-CoV-2.

3257 - Category: IMMUNOPHARMACOLOGY (IP)

Sepsis-induced lipid droplet accumulation enhances antibacterial innate immunity through mechanisms dependent on DGAT-1 and interferon-beta

DUTRA, F.S.P.¹; SANTOS, J.D.C.¹; DE SOUZA, E.K.¹; SAVI, R.V.¹; SOUZA, T.D.S.¹; GIL, H.G.¹; DA CRUZ, G.I.P.¹; SILVA, M.F.D.S.C.²; REIS, P.A.³; BOZZA, P.T.¹. 1. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UFF, RIO DE JANEIRO - RJ - BRASIL; 3. UERJ, RIO DE JANEIRO - RJ - BRASIL.

Lipid droplets (LDs) are lipid-rich organelles recognized as central players in lipid homeostasis, signaling, and inflammation. While their functions in inflammation are well-documented, the mechanisms of LDs in antibacterial immunity and infection resistance remains less understood. Our results show that purified LDs from LPS-stimulated and *E. coli*-infected macrophages exhibited direct *E. coli* anti-bacterial activity. *E. coli*-infection trigger immunometabolic reprogramming and LD accumulation in macrophages. Pharmacological inhibition or genetic knockdown of DGAT1, a key enzyme in triglyceride synthesis, reduced LD formation, bacterial clearance, and pro-inflammatory responses (nitric oxide, PGE₂, CCL2, IL-6). Notably, DGAT1 inhibition impaired the expression of IFN- β and several interferon-stimulated genes (ISGs), including viperin, iNOS, cathelicidin and IGTP, in *E. coli*-infected macrophages. In a cecal-ligation and puncture model of sepsis in C57BL/6 mice, DGAT1 inhibition reduced sepsis-induced LD accumulation in peritoneal cells and decreased levels of IFN- β , CCL2, nitric oxide, and lipid mediators (PGE₂, LTB₄, and RvD1). Furthermore, DGAT1 inhibition accelerated sepsis-related mortality, coinciding with elevated bacterial loads in the peritoneum and bloodstream at 6 and 24 hours post-sepsis. Our results demonstrate that LDs are critical regulators of innate immunity infection resistance, contributing to both bacterial clearance and the coordination of a protective proinflammatory response during sepsis through mechanisms dependent of DGAT-1 and Type I IFN.

Keywords: Lipid metabolism;interferon-beta;sepsis.

3265 - Category: IMMUNOPHARMACOLOGY (IP)

SPECTROSCOPIC AND FUNCTIONAL CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM MOMORDICA CHARANTIA: POTENTIAL FOR ANTITUMOR IMMUNOTHERAPY

COUTO, D.S.; PECONICK, A.P.; DE SOUZA, J.F.F.; KATTAH, H.X.L.; ALVES, E.; OLIVEIRA, E.A.; ALVES, B.H.. UFLA, LAVRAS - MG - BRASIL.

The bitter melon (*Momordica charantia*), a plant from the Cucurbitaceae family, is recognized for its medicinal and pharmacological properties. Although its bioactive compounds remain underexplored, it exhibits promising therapeutic potential against cancer. Raman spectroscopy and spectrophotometric assays for α -amylase were used to identify bioactive compounds and analyze enzymatic inhibition in the fruits and leaves of the plant. The plant material was collected at the Universidade Federal de Lavras. For the dry material, ovens up to 35 °C were used and then subjected to Raman spectroscopic analysis. Spectra were obtained using a confocal Raman system Alpha300 with a 785 nm laser. For the α -amylase spectrophotometric assays, the hydroethanolic extract of the plant was used in microplates with readings at 630 nm, using iodine as a reagent. Phaseolamine was used as the positive control, and results were expressed as a percentage of enzymatic inhibition. The Raman spectra showed peaks at 1000 cm⁻¹ and 1527 cm⁻¹ confirming the presence of phenolic compounds and flavonoids associated with antioxidant activity, 1154 cm⁻¹ polysaccharides, and 784 cm⁻¹ suggesting the presence of protein compounds, with stability between fresh and dried samples, indicating the preservation of bioactive compounds. The *Momordica charantia* extract showed α -amylase inhibition activity, exceeding the standard control by 2.6 times. Among the main targets are the bioactive polysaccharide from *Momordica charantia* (MCBP) and the ribosome-inactivating protein beta-momorcharin. These components are related to anticancer and immunomodulatory activity. Parallel studies with *in vitro* tests using HeLa cell lines and *in silico* predictions investigate the apoptotic mechanisms and bioactive functions of the extracts. The results reinforce the potential of *M. charantia* compounds as a basis for the development of future immunotherapies and applications in pharmacognosy.

Keywords: Pharmacognosy;Cancer;Bioactives.

3275 - Category: IMMUNOPHARMACOLOGY (IP)

In Silico Characterization of Putative Evasins from *Rhipicephalus microplus* ticks with Anti-inflammatory Potential

BRUNATO, M.L.; DE MORAES, A.; DE SOUZA, I.S.; FERREIRA, B.R.. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - USP, RIBEIRÃO PRETO - SP - BRASIL.

In this study, we performed the in silico functional characterization of two putative evasins, Bm-nr-265974 and Bm-nr-167608, identified in the sialotranscriptome of the tick *Rhipicephalus microplus*. These proteins will be subsequently evaluated as potential anti-inflammatory drug candidates.

The selection of proteins of interest was focused on proteins expressed and secreted in *R. microplus* saliva. The identification of sequences with the highest evolutionary proximity and amino acid conservation relative to Evasin-3 from *Rhipicephalus sanguineus* ticks, which was previously characterized, was carried out using the BLAST tool. Highly similar sequences were subjected to three-dimensional modeling, employing the Robetta platform, via the de novo synthesis method.

Of the 36 putative evasins initially analyzed, two were identified as homologous to Evasin-3: Bm-nr-265974 (30% identity) and Bm-nr-167608 (30% identity). The amino acid sequence alignment revealed conservation of residues among the three proteins, particularly highlighting the positions of the six cysteine residues, which are crucial for chemokine-binding functional activity. The three-dimensional modeling corroborates this observation, indicating the proximity of cysteine residues in the predicted structure and, consequently, the presence of a predicted functional area for interaction with chemokines.

In summary, the in silico characterization of putative evasins Bm-nr-265974 and Bm-nr-167608 revealed global similarity and conservation within the functional cysteine-rich region, akin to the already characterized Evasin-3. These findings suggest the preservation of function among the sequences and a possible chemokine-inhibiting anti-inflammatory potential for the proteins under investigation, which will require validation through in vitro and in vivo assays.

Keywords: Ticks ;Evasins ;bioinformatics.

3279 - Category: IMMUNOPHARMACOLOGY (IP)

Evaluation of metabolic reprogramming and lipid droplet accumulation in the heart during experimental sepsis and its consequences on long-term cardiovascular changes

SOUZA, T.D.S.¹; PIRES, A.D.S.²; TERRA, E.³; DOS SANTOS, P.D.M.A.³; REIS, P.A.³; PEREIRA-DUTRA, F.¹; BOZZA, P.T.¹. 1. INSTITUTO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO (UNIFESP), SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE ESTADUAL DO RIO DE JANEIRO (UERJ), RIO DE JANEIRO - RJ - BRASIL.

Sepsis is a life-threatening organ dysfunction caused by the host's dysregulated immunometabolic response to infection, and is one of the leading causes of death in intensive care units. Survival in sepsis does not necessarily equate to restoration of quality of life or improved outcomes for survivors. Cardiovascular changes are among the most frequent long-term manifestations. The mechanisms behind these complications are not yet fully understood, but studies suggest that systemic inflammatory response and metabolic changes contribute to cardiac dysfunction. Studies show lipid metabolism as the main metabolic change in septic patients, including increased accumulation of lipid droplets (LD) in various tissues. LD are central structures in the lipid and energy homeostasis of cells. In this context, the dysregulation of lipid metabolism and LD in the cardiac pathophysiology of sepsis still needs further investigation. Based on this, our hypothesis in this study is that metabolic reprogramming and LD accumulation in the myocardium contribute to the cardiac changes observed in sepsis and its long-term consequences. Our objective in this study is to characterize and evaluate changes in lipid metabolism and LD accumulation in cardiac pathophysiology in experimental sepsis. To this end, male C57BL/6 mice underwent cecal ligation and puncture to induce experimental sepsis, and the heart was removed for analysis of changes in lipid metabolism in cardiac tissue induced by experimental sepsis at 24 hours, 48 hours, and 15 days. Our data indicate that sepsis induces increased expression of the main genes associated with lipid metabolism at 48 hours and 15 days. In addition, the cardiac inflammatory response induced by experimental sepsis appears to be compartmentalized in the atrium and not in the whole heart. Our results indicate that reprogramming of lipid metabolism occurs in the hearts of animals subjected to sepsis both in the acute phase and later.

Keywords: Lipid droplets;Sepsis;cardiovascular changes.

3319 - Category: IMMUNOPHARMACOLOGY (IP)

Novel Immunopathological Insights into Africanized Honeybee Envenomation: Emphasis on Erythrocyte-Derived DAMPs and Complement System Activation

DE FRANÇA, F.S.¹; GABRILI, J.J.M.²; LEONEL, T.B.¹; ORSI, R.D.O.³; LAMBRIS, J.D.⁴; WOODRUFF, T.⁵; TAMBOURGI, D.V.¹. 1. BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL; 2. BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL; 3. UNESP - BOTUCATU, BOTUCATU - SP - BRASIL; 4. UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA - ESTADOS UNIDOS DA AMÉRICA; 5. THE UNIVERSITY OF QUEENSLAND, BRISBANE - AUSTRALIA.

Envenomation by Africanized honeybee (*Apis mellifera*) swarms is increasingly common in Brazil and poses a significant life threat due to its capacity to trigger complex pathological responses. The hallmark complication is disseminated intravascular hemolysis, closely linked to acute renal failure in affected patients. Clinical observations also reveal diverse inflammatory events post-envenomation, yet the mechanisms underlying these immunological reactions remain poorly understood. The role of erythrocyte lysis in envenomation immunopathogenesis is insufficiently characterized. Using an *ex vivo* human whole blood model (HWB) combined with immunochemical methods, we demonstrated that *A. mellifera* venom (AmV) induces substantial erythrocyte injury, evidenced by elevated hemolysis indices and increased extracellular hemoglobin (Hb) and free heme levels. The HWB model showed intense complement system activation, indicated by altered pathway activity and raised levels of Ba fragment, C1s/C1 inhibitor complex, anaphylatoxins C3a, C4a, C5a, and the terminal sC5b-9 complex. Additionally, AmV stimulated release of inflammatory lipid mediators including LTB₄, PGE₂, TXB₂, and chemokines CCL2, CCL5, CXCL8, CXCL9, and CXCL10. The modulatory roles of Hb and free heme were assessed using their natural scavengers, haptoglobin and hemopexin, both effectively reversing complement abnormalities and reducing chemokine and eicosanoid release. Complement activation was a central inflammatory event strongly influenced by Hb and free heme, prompting mechanistic evaluation. Targeted complement inhibition with peptides Cp40 (C3/C3b antagonist) and PMX205 (C5aR1 antagonist) significantly reduced CXCL8 production and lipid mediator synthesis. Collectively, these findings establish AmV as a potent immunopathological agent that triggers inflammatory cascades partly mediated by erythrocyte-derived DAMPs and complement activation, substantially contributing to the pathogenesis of envenomation.

Keywords: *Apis mellifera* venom; Complement System; Erythrocytes-derived DAMPs.

3322 - Category: IMMUNOPHARMACOLOGY (IP)

Cnidoscolus urens modulates effector functions of human neutrophils

ALBIERO, L.R.¹; LIMA, V.D.S.²; EMERICK, G.L.¹; SINHORIN, V.D.G.¹. 1. UFMT, SINOP - MT - BRASIL; 2. UFMT, CUIABÁ - MT - BRASIL.

Introduction: *Cnidoscolus urens* (*C. urens*; cansanção) has been used in folk medicine for its anti-inflammatory applications. The present study aimed to investigate the immunomodulatory effects of hydroethanolic extracts of *C. urens* leaves on the functions of peripheral neutrophils of healthy humans, regarding their oxidative metabolism and degranulation. **Objective:** in vitro evaluation of *C. urens* extracts, under the modulation of the effector functions of peripheral neutrophils from healthy humans. **Material and methods:** Neutrophils were treated with *C. urens* (IC₅₀ - 5 mg/mL) and oxidative metabolism was evaluated by the NBT assay stimulated by zymozan, or zymozan opsonized with human serum in a spectrophotometer by measuring the production of superoxide anion (O₂^{•-}) and read in a spectrophotometer (565 nm); evaluation of myeloperoxidase activity by the TMB reduction assay through the production of HOCl and read in a spectrophotometer (630 nm); and elastase degranulation was evaluated, stimulated with fMLP. Elastase enzyme activity was assessed through the degradation of the SAAVNA substrate and read in a spectrophotometer at 405 nm. (CEP/UFMT/Sinop Opinion: 7,430,150). For statistical analysis, the Mann-Whitney test or one-way ANOVA was performed with a significance level set at $p < 0.05$. **Results:** *C. urens* modulated superoxide anion by NBT by approximately 90%, modulated MPO activity by 66% and modulated the elastase degranulation process by 50%. **Conclusion:** *C. urens* negatively modulates the effector functions of neutrophils, contributing to a potent therapeutic effect for inflammatory diseases such as autoimmune diseases, where neutrophils play an important role.

Keywords: Neutrophils; *Cnidoscolus urens*; inflammation.

3333 - Category: IMMUNOPHARMACOLOGY (IP)

RIPK2 Signaling Negatively Regulates the IL-23/IL-17 Axis in Psoriasis

CONCEIÇÃO, M.L.P.; DE MELO, B.M.S.; MARTINS, T.V.; DE OLIVEIRA, C.V.; DA SILVA, G.V.L.; PÚBLIO, G.A.; MONTEIRO, G.R.C.; DAMASCENO, L.E.A.; CUNHA, T.M.; CUNHA, F.Q.; ALVES FILHO, J.C.F. USP, RIBEIRÃO PRETO - SP - BRASIL.

Psoriasis is a systemic inflammatory skin disease characterized by keratinocyte hyperproliferation and immune cell infiltration, primarily driven by the IL-17/IL-23 axis. RIPK2 is a key adaptor in NOD-like receptor signaling and a well-known regulator of intestinal inflammation. However, its role in the pathophysiology of psoriasis remains poorly understood. To investigate this, we performed single-cell RNA sequencing (scRNA-seq) analyses using publicly available datasets from skin samples of psoriatic patients and mice with experimental psoriasis. We found increased RIPK2 expression specifically in dermal dendritic cells (DCs). To explore the functional relevance of this finding, we induced experimental psoriasis using the imiquimod (IMQ) model in RIPK2-deficient mice (*Ripk2*^{-/-}). Unexpectedly, *Ripk2*^{-/-} mice developed a more severe form of psoriasis compared to wild-type controls, characterized by elevated expression of K17 and IL-23 in the skin, as well as an increased frequency of IL-17-producing CD4⁺ and γδ T cells in the lymph nodes. Moreover, RIPK2 deficiency led to enhanced production of IL-6, TNF-α, and IL-23 by bone marrow-derived dendritic cells (BMDCs), along with upregulation of activation markers such as CD80 and CD86 following stimulation with LPS and S100A9. Interestingly, co-culture experiments revealed that RIPK2^{-/-} DCs promoted greater T cell proliferation and IL-17 production compared to WT DCs. Together, these findings indicate that RIPK2 signaling negatively regulates the IL-23/IL-17 axis in the skin, playing a critical role in the immunomodulation of psoriasis. This pathway may represent a novel target for elucidating the mechanisms underlying psoriasis progression.

Keywords: psoríase;células dendríticas;NOD2/RIPK2

3335 - Category: IMMUNOPHARMACOLOGY (IP)

Immunomodulatory effects of kaempferide isolated from caatinga propolis on human monocytes

DE CARVALHO, Y.B.R.; NASTARO, P.; SARTORI, A.A.; SFORCIN, J.M.. INSTITUTO DE BIOCÊNCIAS DE BOTUCATU - IBB UNESP, BOTUCATU - SP - BRASIL.

In the last few years, natural products have been subject of study by the pharmaceutical industry in search of new drugs. With this new interest from the industry in these compounds, due to the presence of new technologies that allow their study, the scientific community has also intensified its studies on these materials, seeking to characterize their chemical, physical and biological properties. Among these products is kaempferide, an o-methylated flavonol abundantly present in a series of plant and apicultural sources, such as propolis from the northeastern caatinga produced from *Mimosa tenuiflora* (Willd.) Poir., which has been widely studied as a possible source of new bioactive compounds for the production of new drugs. This work therefore analysed the viability of human monocytes after incubation with kaempferide and the production of pro- and anti-inflammatory cytokines or those related to the inflammasome (TNF-α, IL-1β, IL-6, IL-10, IL-18, IL-33). The substance was evaluated after incubation with lipopolysaccharide (LPS) for the production of arachidonic acid metabolites (PGE2 and LTB4), as well as for the production of reactive oxygen species (ROS) and membrane receptors (CD62L, CD80, HLA-DR, TLR2 and TLR4).

The results revealed a pró-inflammatory profile in human monocytes after stimulation with kaempferide without causing cytotoxicity. This profile is evidenced by the higher levels of pro-inflammatory cytokines, ROS, and bactericidal activity in treated cells when compared to the control group. PGE2 was lower in treated cells when compared to the control group, but it doesn't changed cytokine production as shown by TNF-α level. TLR2 expression seemed up-regulated, although no significance was attested ($p > 0.05$).

This is one of the few works to evaluate kaempferide effects on isolated cells, and the first to analyze it on monocytes, opening new paths to a better understanding about kaempferide immunomodulatory effects.

Keywords: Kaempferide;Pharmacology;Inflammation.

3341 - Category: IMMUNOPHARMACOLOGY (IP)

ANTILEISHMANIAL AND IMMUNOMODULATORY ACTIVITY OF FERAI, A BROMINATED CHALCONE: IN VITRO STUDY

DOS SANTOS, B.C.¹; UZÊDA, L.F.A.¹; SANTOS, F.D.S.¹; SANTANA, R.P.D.J.¹; SILVA, G.R.²; MAGALHÃES, H.I.F.²; GUERRA, F.S.²; SOARES, M.B.P.³; GUIMARÃES, E.T.¹. 1. UNIVERSIDADE DO ESTADO DA BAHIA, SALVADOR - BA - BRASIL; 2. UNIVERSIDADE FEDERAL DA PARAÍBA, JOÃO PESSOA - PB - BRASIL; 3. INSTITUTO GONÇALO MUNIZ FIOCRUZ-BA, SALVADOR - BA - BRASIL.

Introduction: Leishmaniasis is a global public health concern. Current treatments for the disease have several associated limitations. Previous studies from our research group have demonstrated that FERAI is effective against both the promastigote and amastigote forms of *Leishmania braziliensis*, inducing significant ultrastructural alterations in the parasite. In this study, the antileishmanial and immunomodulatory activities of FERAI, a brominated chalcone, were evaluated through *in vitro* experimental assays. The aim was to determine the nitric oxide and cytokine production profiles in macrophages infected with *Leishmania braziliensis* and/or stimulated with lipopolysaccharide (LPS).

Methods: Cytokine and nitric oxide (NO) levels were measured in the supernatants of macrophages infected with *L. braziliensis* and/or stimulated with LPS, using ELISA and the Griess reaction. Statistical analysis was performed using GraphPad Prism software. **Results:** After 4 hours of treatment, FERAI inhibited TNF- α production in the group infected with *L. braziliensis*, compared to the negative control. After 24 hours, NO production was increased in all treated groups, with a particularly notable elevation in the infected and LPS-stimulated group treated with 10 μ M, compared to the untreated group. Suppression of the pro-inflammatory cytokines IL-1 β , IL-6, and IL-12 was observed after 48 hours of treatment with FERAI at both 10 μ M and 5 μ M concentrations. Furthermore, an increase in the production of the anti-inflammatory cytokines IL-10 and TGF- β was detected in the group treated with 10 μ M FERAI, compared to the untreated group. **Conclusion:** FERAI exhibits significant activity against *L. braziliensis* at non-toxic concentrations and modulates cytokine production in stimulated macrophages. These effects suggest an immunomodulatory potential capable of interfering with the pathogenesis of cutaneous leishmaniasis. Further studies are currently underway to elucidate the underlying mechanisms.

Keywords: Leishmania;Immunomodulation;Chalcone.

3354 - Category: IMMUNOPHARMACOLOGY (IP)

Effects of supplementation with Spirulina in natura in rats with overfeeding-induced obesity during lactation: sex-specific analysis

STRAUSS, G.D.F.; ANDREAZZI¹, A.E.; ², ¹M.M.F.; DE MEDEIROS³, V.P.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Natural *spirulina* has recently emerged as a promising dietary supplement due to its antioxidant, anti-inflammatory, antitumor, immunomodulatory, and antibacterial properties. It has been widely used over the last years. In addition to improving individual metabolic parameters—such as food intake and adiposity—this study aimed to investigate the transgenerational effects of natural spirulina supplementation during lactation on body weight, food intake, and adiposity, of rats, focusing on the F1 generation. Animals were divided into two groups: F1CV and F1CS (CEUA/UFJF Protocol 025/2023). The F1CV group consisted of offspring from the F0 generation that received an oral saline solution, while the F1CS group consisted of offspring whose F0 generation received natural *spirulina* (400 mg/kg) diluted in saline. Feed intake was measured three times per week. And body weight was measured once a week for 30 days, both after weaning. Final body weight, perigonadal, and retroperitoneal fat were collected and weighed during euthanasia. Statistical analysis was conducted using GraphPad Prism v.10.1. Results indicated a significant reduction in food intake and retroperitoneal fat in the F1CS group compared to the F1CV group, as well as reduced perigonadal fat in F1CS males. These findings suggest that *spirulina* supplementation during lactation can effectively reduce food consumption and adiposity, supporting its potential as an affordable and accessible strategy for metabolic regulation.

Keywords: Spirulina;Obesity; ;transgenerational effects.

3414 - Category: IMMUNOPHARMACOLOGY (IP)

Pomegranate Seed Oil and Nanostructured Lipid Carriers: Assessing Immunomodulatory Properties

SILVA, J.L.S.¹; DE OLIVEIRA, R.C.²; PINTO, N.V.³; CAVALCANTE, D.N.⁴; SANTOS, F.G.P.⁵; MEMÓRIA, K.A.S.⁵; CAVALCANTE, T.T.A.⁵; NETO, J.A.C.²; BEZERRA, J.D.A.⁴; DA COSTA, A.G.²; CARNEIRO, G.³; FARIA, J.A.Q.A.⁵. 1. UNIVERSIDADE FEDERAL DO AMAZONAS, MANAUS - AM - BRASIL; 2. FUNDAÇÃO HOSPITALAR E HEMATOLOGIA E HEMOTERAPIA DO AMAZONAS (FHEMOAM), MANAUS - AM - BRASIL; 3. UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI (UFVJM), DIAMANTINA - MG - BRASIL; 4. INSTITUTO FEDERAL DO AMAZONAS (IFAM), MANAUS - AM - BRASIL; 5. UNIVERSIDADE FEDERAL DO AMAZONAS (UFAM), MANAUS - AM - BRASIL.

Introduction: Driven by the search for new breast cancer therapies, research has delved into natural products like pomegranate (*Punica granatum*), given that its compounds exhibit immunomodulatory activity. To optimize the action of these compounds, nanostructured lipid carriers (NLCs) were used, which increase solubility and facilitate controlled drug release. This study focused on the chemical characterization and evaluation of the immunomodulatory properties of pomegranate seed oil (PSO)-loaded NLCs in macrophage-differentiated THP-1 cells. **Methods:** PSO was processed by transesterification and analyzed by gas chromatography coupled to mass spectrometry. Functional assays were performed on PMA-differentiated human THP-1 macrophages treated with PSO, medium-chain triglycerides (MCT), PSO-loaded NLCs, and MCT-loaded NLCs. Intracellular reactive oxygen species (ROS) levels were assessed using the probe 2',7'-dichlorofluorescein diacetate. The cytokine profile was quantified using a BD Cytometric Bead Array Human Th1/Th2/Th17 cytokine kit from the supernatant of macrophages previously treated for 24 hours and then stimulated with lipopolysaccharide at 1 mg/mL for additional 24 hours. **Results:** The chemical profile identified 12 main compounds in PSO, with linolenic acid being the most predominant (51.18%), linoleic acid (7.21%), elaidic acid (7.25%), and isomers of linolenic acid corresponding to 26.45%. Our findings showed that PSO-loaded NLCs (NLC-PSO) significantly increased ROS levels upon H₂O₂-induced oxidative stress, whereas PSO treatment alone exhibited a reduction in ROS levels. Furthermore, PSO treatment alone increased IL-10 cytokine levels, while the NLC-PSO nanoformulation promoted an increase in pro-inflammatory cytokines, specifically IL-6 and TNF- α . **Conclusion:** Our results indicate that PSO exhibits varied responses depending on its form of administration, suggesting that the delivery system is a critical factor in modulating the cellular response.

Keywords: immunopharmacology;immunomodulation;lipid nanocarriers.

3422 - Category: IMMUNOPHARMACOLOGY (IP)

Topical SCFAs enhance skin wound healing in diabetic mice via histone acetylation in a FFAR2-independent manner

RIBEIRO, W.R.; DE MENEZES, M.H.T.; FRAGA JUNIOR, V.D.S.; SILVA, J.K.A.D.O.; DE OLIVEIRA, A.C.D.S.C.; BENJAMIM, C.F.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Chronic wounds, characterized by persistent inflammation and impaired tissue repair, are a major complication of type 1 diabetes mellitus. Current treatments remain limited, underscoring the need for novel therapeutic strategies. Short-chain fatty acids (SCFAs), such as propionate and butyrate, have emerged as potential modulators of inflammation and tissue regeneration by acting through G-protein coupled receptors, such as FFAR2 and epigenetic mechanisms, including inhibition of histone deacetylase (HDAC) activity. This study aimed to investigate the therapeutic effect of topical treatment with propionate and butyrate on wound healing using a pharmacologically induced type 1 diabetes model with alloxan in C57BL/6J mice. Topical propionate (30 mM) and butyrate (100 mM) treatments significantly accelerated wound closure in diabetic mice compared to untreated controls ($P < 0.05$). On day 7 post-injury, butyrate-treated mice showed reduced IL-6, MIP-2, and TIMP-1 levels ($P < 0.05$), while both treatments decreased TGF- β and MMP-3 expression ($P < 0.05$). SCFA treatment increased HIF-1 α , HDAC3, HDAC8, and HDAC9 gene expression ($P < 0.05$), without altering FFAR2 levels. These SCFAs also accelerated wound closure in diabetic FFAR2 knockout mice ($P < 0.05$), indicating a FFAR2-independent mechanism. Enhanced histone H3K27 acetylation was observed in treated animals, supporting an epigenetic mode of action. In vitro, propionate and butyrate promoted keratinocyte and fibroblast migration, including under pro-inflammatory conditioned media, suggesting direct effects on epithelial and stromal cell function. These findings indicate that topical SCFAs improve diabetic wound healing through epigenetic modulation and stimulation of cell migration, independent of FFAR2 signaling, highlighting their potential as novel therapies for chronic diabetic wounds.

Keywords: scfa;skin wound healing;diabetes.

3423 - Category: IMMUNOPHARMACOLOGY (IP)

Differential Toll-like Receptor Activation by Serum from Patients with mood disordersDE FARIAS, I.S.; FIDALGO, T.M.; HAYASHI, M.A.F.. FEDERAL UNIVERSITY OS SÃO PAULO - UNIFESP, SP - SP - BRASIL.

Depressive symptoms such as low energy, sadness, and loss of interest are hallmark features of mood disorders, including major depressive disorder (MDD) and bipolar disorder (BD). These are chronic, disabling conditions with complex etiologies and a significant global burden. The neuroimmunological hypothesis suggests that low-grade systemic inflammation plays an active role in the onset and progression of these disorders by impairing synaptic function, neuroplasticity, and neuronal integrity. Toll-like receptors (TLRs), key components of innate immunity, mediate peripheral-central immune communication and have been increasingly implicated in psychiatric pathophysiology. TLR activation is essential for the body's defense against pathogens, triggering a signaling cascade that leads to the production of pro-inflammatory cytokines and activation of immune cells. Although evidence suggests that TLRs are involved in immuno-metabolic regulation and CNS homeostasis, the pattern of their activation by circulating factors remains poorly defined. Here, we evaluated whether serum from medicated MDD and BD patients differentially activates TLRs. Using HEK-Blue reporter cells expressing individual human TLRs, we found that MDD serum selectively increased TLR2/6 activation, while BD serum enhanced both TLR2/6 and TLR7 compared to healthy controls (HCs). In contrast, TLR4 and TLR5 were significantly less activated by both mood disorder sera than by HCs. All patients were using antidepressants and/or antipsychotics, which may also influence immune signaling, since selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, modulate pro-inflammatory cytokines via NF- κ B and cAMP/PKA pathways. Thus, the observed TLR activation may result from a combination of disease-related immune changes and pharmacological effects. These findings support the hypothesis that dysregulated immune signaling contributes to the neurobiology and progression of mood disorders.

Keywords: Major Depressive Disorder;Bipolar Disorder;Toll-like receptors (TLR).

3482 - Category: IMMUNOPHARMACOLOGY (IP)

SREBPs mediate lipid metabolic reprogramming and contribute to ZIKV-induced cellular alterationsDIAS, S.D.S.G.; RAMOS, D.; SOARES, V.C.; AZEVEDO-QUINTANILHA, I.G.; SANTOS, J.D.C.; SOUZA, T.M.L.; BOZZA, P.T.. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Zika virus (ZIKV) infection poses a global public health challenge due to its association with neurological complications in adults and congenital syndromes in newborns. ZIKV exhibits a tropism for neural cells, disrupting development and inducing cell death. Like many RNA viruses, ZIKV manipulates host lipid metabolism and utilizes lipid droplets (LDs) to support its replication. While LDs are known for their roles in lipid storage and cellular homeostasis, the formation and function of LDs during ZIKV infection in neural cells remain poorly understood. Here, we investigated the involvement of the transcription factor Sterol Regulatory Element-Binding Protein (SREBP) in lipid metabolism during ZIKV infection in human SH-SY5Y neural progenitor cells. Using both pharmacological inhibition with Betulin and gene silencing of SREBP-1 and SREBP-2, we evaluated the impact of SREBP activity on lipid metabolism and virus-induced cellular dysfunction. Our results show that ZIKV infection enhances SREBP-1 activation and upregulates key lipid metabolism proteins, including PLIN-2, DGAT-1, and FASN, promoting LD accumulation. Inhibition of SREBP processing reduced LD biogenesis, downregulated these proteins, and decreased viral replication. Gene silencing experiments corroborating the role of SREBPs in supporting ZIKV replication. Additionally, infected neural cells exhibited increased ROS production and mitochondrial dysfunction, both of which were alleviated by SREBP inhibition, alongside reduced caspase-1 activation and protection from virus-induced cell death. These findings highlight SREBPs as key regulators of lipid metabolic remodeling that support ZIKV replication and pathogenesis, offering potential targets for host-directed antiviral therapies.

Keywords: Lipid metabolism;Oxidative stress;Zika virus.

3490 - Category: IMMUNOPHARMACOLOGY (IP)

SARS-CoV-2-induced platelet activation enhances viral replication in pulmonary epithelial cells through the thromboxane pathway

DE CAMPOS, M.M.¹; AZEVEDO-QUINTANILHA, I.G.¹; DIAS, S.D.S.G.²; SANTOS, J.D.C.²; OLIVEIRA, D.M.¹; GONCALVES, R.M.¹; SOARES, V.C.¹; SOUZA, T.M.L.E.²; HOTTZ, E.³; BOZZA, F.A.²; BOZZA, P.T.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. UFJF, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Platelets are well recognized for their essential role in hemostasis and also participate in innate immunity and inflammatory responses. In COVID-19, platelet activation contributes to thromboinflammation; however, their interaction with pulmonary epithelial cells remains poorly explored. **Objectives:** To characterize platelet activation and its interaction with SARS-CoV-2-infected Calu-3 cells, investigating the immunometabolic mechanisms involved.

Methods: Calu-3 cells (ATCC HTB-55) were infected with SARS-CoV-2 and subsequently stimulated with platelets from healthy donors or with U46616. The interaction was analyzed after 24 and 48 hours, considering platelet activation parameters, production of inflammatory mediators, and viral load. **Results/Discussion:** Although platelets are non-permissive to viral replication, their interaction with infected Calu-3 cells led to platelet activation, as evidenced by increased expression of CD62P and CD63 markers, and a significant increase in SARS-CoV-2 replication. This effect was associated with elevated production of thromboxane A2 (TXA2) and tissue factor, both indicators of platelet activation and aggregation. Inhibition of TXA2 synthesis reduced viral replication, suggesting this pathway contributes to creating a permissive microenvironment for infection. Furthermore, the agonist U46619, a stable TXA2 analog, mimicked the effect of platelets by enhancing viral replication, reinforcing the central role of TXA2 signaling in this process. A decrease in IFN- α production was also observed in infected cells stimulated with platelets, indicating platelet stimulation may suppress cellular antiviral mechanisms. **Conclusion:** Platelets negatively modulate the antiviral response in SARS-CoV-2-infected pulmonary epithelial cells, promoting viral replication through thromboxane A2 pathway activation and IFN- α suppression. Pharmacological modulation of this pathway was effective in reducing viral load.

Keywords: platelets; SARS-CoV-2; thromboxane A2.

3499 - Category: IMMUNOPHARMACOLOGY (IP)

Enhancing Natural-Based Acne Treatments: The Role of Co-Stabilizers in β -Caryophyllene Encapsulation in PCL Nanoparticles

CORDEIRO, A.P.; MARTINS, A.L.; DE ARAÚJO, P.H.H.; SAYER, C.. UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRASIL.

Acne is a chronic inflammatory skin condition affecting 9.4% of the global population. Current treatments include retinoids, antibiotics, benzoyl peroxide, and hormonal therapies, but they often cause issues like antibiotic resistance, and unpleasant side effects. This has increased the interest in natural bioactive compounds such as β -caryophyllene (β CF), known for its anti-inflammatory and antimicrobial properties. Nanoparticles enhance acne treatment by improving active substance release, penetration, and residence time in the skin. Thus, the aim of this work was to investigate the effects of the co-stabilizers (CS) over β CF encapsulation in Poly(ϵ -caprolactone) (PCL) nanoparticles (PCL- β CF-NP), as well the anti-inflammatory activity of the β CF loaded in PCL-NP. The PCL- β CF-NP were prepared combining the miniemulsification and solvent evaporation techniques using β CF, Crodamol, or Copaiba Oil as CS. All the formulations had encapsulation efficiency > 95%. PCL- β CF-NP prepared using Crodamol and Copaiba Oil as CS presented submicrometric size (< 200 nm), narrow size distribution (PDI < 0.2), and zeta potential $\geq$ -30 mV. However, the use of β CF itself as CS negatively affects PCL- β CF-NP, increasing the size (> 200 nm) and distribution (PDI > 0.2), and decreasing zeta potential to -10 mV. The anti-inflammatory activity of PCL- β CF-NP was accessed measuring the capacity of the formulations to decrease the levels of IL-1 β , IL-6, and IL-10 in murine macrophages. The use of Crodamol as CS enhanced the anti-inflammatory activity of the formulation compared to β CF. Furthermore, the use of Copaiba oil as CS presented promising results by decreasing the minimum bactericidal concentration of PCL- β CF-NP in *Propionibacterium acnes*. These results show the potential of PCL-NPs to effectively carry β CF for developing natural-based acne treatments. Reducing β CF in the formulation could improve the system stability and availability, enhancing the delivery of the bioactive compound.

Keywords: Polymeric nanoparticles; Drug Encapsulation; Anti-Inflammatory Activity.

3514 - Category: IMMUNOPHARMACOLOGY (IP)

Advances in understanding the efficacy of single-domain antibodies in mitigating local tissue damage induced by Bothrops snake envenomation: progress in antivenom immunotherapy

PRADO, N.D.R.¹; ROBERTO, S.A.¹; DE MELO, T.F.¹; DA SILVA, M.A.²; FIALHO, S.N.²; DE HOLANDA, K.E.R.³; PALOSCHI, M.V.¹; FERNANDES, C.F.C.⁴; ZULIANI, J.P.¹; PEREIRA, S.D.S.¹. 1. FUNDAÇÃO OSWALDO CRUZ RONDÔNIA, FIOCRUZ RONDÔNIA, PORTO VELHO - RO - BRASIL; 2. INSTITUTO FEDERAL DE RONDÔNIA, IFRO, PORTO VELHO - RO - BRASIL; 3. UNIVERSIDADE FEDERAL DO AMAZONAS, UFAM, PORTO VELHO - RO - BRASIL; 4. FUNDAÇÃO OSWALDO CRUZ CEARÁ, FIOCRUZ CEARÁ, PORTO VELHO - RO - BRASIL.

Camelid single-domain antibodies (VHHs) are highly stable, antigen-specific immunobiologics with versatile therapeutic potential. In the context of snakebite envenomation, they offer a promising approach to overcome key limitations of conventional antivenoms, particularly the poor neutralization of local tissue damage. Amid, VHHs directed against key toxins, such as phospholipase A₂ (PLA₂), have gained prominence due to their specific neutralization capacity and favorable pharmacological profile. This study evaluated the neutralizing potential of a monomeric anti-PLA₂ VHH in a post-envenomation murine model and investigated its pharmacological behavior through ex vivo percutaneous penetration studies. In vivo, mice envenomed with isolated PLA₂ toxin or Bothrops jararacussu venom were treated with VHH at a 1:10 ratio (p/p), and myotoxicity markers (CK and LDH) and leukocyte influx (intravital microscopy) were assessed. For the ex vivo assay, porcine auricular skin (Sus scrofa domesticus) in Franz diffusion cells received anti-PLA₂ VHH in PBS, VHH in an oil-in-water microemulsion, or PBS control. Receptor compartment samples were collected hourly for 12h, analyzed by UV-Vis spectrophotometry, and, after extraction, the retained VHH in skin was quantified. In vivo, VHH treatment significantly reduced myotoxicity, lowering serum CK and LDH by 60% after B. jararacussu envenomation, and LDH by 60% and 80% following BthTX-I and II exposure, respectively. Leukocyte influx induced by BthTX-I decreased by 28% as shown by intravital microscopy. In percutaneous penetration assays, VHH in PBS showed rapid release (81.76% within 1 h), while the microemulsion provided a more controlled profile (35.87% at 1 h; 76.74% at 6 h). No skin retention was observed after 12 h. VHH showed strong neutralization of B. jararacussu venom and toxins, with PBS enabling rapid release for early PLA₂ neutralization, supporting its use in topical delivery to overcome antivenom limitation.

Keywords: Neutralization;local damage;Antivenom.

2640 - Category: IMMUNOREGULATION (IR)

Analysis of the modulation of dendritic cells by extracellular vesicles from the blood of patients with chronic lymphocytic leukemia

ALVES, C.R.B.; DE CAMPOS, C.L.V.; SILVA, A.E.N.E.; VIEIRA, M.S.; REIS, D.S.; PEREIRA, M.H.G.; XAVIER, L.D.A.; SABINO, A.D.P.; SANTIAGO, H.D.C.. UFMG, BELO HORIZONTE - MG - BRASIL.

Introduction: Chronic lymphocytic leukemia (CLL) is a heterogeneous and incurable disease characterized by the accumulation of dysfunctional mature B lymphocytes. One of the immune-modulating mechanisms of CLL is the release of extracellular vesicles (EVs), which carry biomolecules capable of altering immune cells and promoting an immunosuppressive microenvironment. **Objective:** To evaluate the impact of EVs from CLL patients and healthy donors (HD) on the maturation and function of monocyte-derived dendritic cells (mo-DCs). **Methodology:** PBMCs were obtained from CLL patients and HD. B lymphocytes from HD and PBMCs from patients (mainly leukemic B cells) were cultured for 48 hours. EVs were isolated from the supernatants by ultracentrifugation. Monocytes from HD were differentiated into DCs with GM-CSF and IL-4 in the presence of EVs for 5 days and activated with LPS for 48 hours. Flow cytometry was used to analyze the phenotype, and functional assays were performed by co-culturing mo-DCs with allogeneic T lymphocytes. **Results:** EVs from CLL patients increased CD11c+CD14+ mo-DCs (P=0.0281) and decreased CD11c+CD14- (P=0.0171) compared to EVs from HD. After LPS activation, there was a reduction in the frequency of CD86, and MFI of HLA-DR, CD80, and CD86 was also decreased, indicating a tolerogenic phenotype. These mo-DCs induced decreased IFN- γ (P=0.0373) and TNF (P=0.0109) production by CD4+ T cells, and IFN- γ (P=0.0084) and TNF (P=0.019) by CD8+ T cells. Additionally, there was an increase in IL-10 production by CD4+ (P=0.0303) and the differentiation of IL-10-producing regulatory T cells (P=0.0443). **Conclusion:** EVs from CLL patients induce the differentiation of mo-DCs with a tolerogenic phenotype, impairing the activation of effective antitumor immune responses.

Keywords: Leukemia;t regulatory;Dendritic.

2645 - Category: IMMUNOREGULATION (IR)

Environmental Influence on Immunosenescence Signatures

COELHO, M.M.¹; SOARES, B.F.S.¹; MOREIRA, F.C.¹; ZUCCHERATO, L.W.¹; RAMIREZ, J.A.Z.²; VENTURA, L.H.D.A.¹; TORRES, L.¹; CAMATTA, G.C.¹; DURSO, D.F.¹; COSTA, M.S.¹; SATO, H.I.¹; GUIMARÃES, H.C.³; BARBUTO, R.C.⁴; VEIGA, A.P.R.⁵; ATAÍDE, N.⁵; CAETANO, G.P.⁵; RANGON, S.⁵; SOUZA, R.⁵; CAÇADOR, M.A.²; SALGADO, C.L.²; TUPINAMBÁS, U.¹; CARVALHO, A.T.⁶; TEIXEIRA, S.M.R.¹; DA FONSECA, D.M.²; MAIOLI, T.U.¹; FARIA, A.M.C.¹. 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL; 3. HOSPITAL RISOLETA TOLENTINO NEVES, BELO HORIZONTE - MG - BRASIL; 4. HOSPITAL RISOLETA TOLENTINO, BELO HORIZONTE - MG - BRASIL; 5. INSTITUTO DE INFECTOLOGIA EMILIO RIBAS, SÃO PAULO - SP - BRASIL; 6. INSTITUTO RENE RACHOU, FUNDAÇÃO OSWALDO CRUZ, BELO HORIZONTE - MG - BRASIL.

Introduction: Individuals of the same chronological age may exhibit different biological or epigenetic ages, influenced by environmental factors. Assuming that variations in antigen exposure—driven by geographical differences—may be associated with distinct rates and patterns of biological aging, this study aimed to compare immunosenescence signatures in individuals residing in Belo Horizonte (BH) and São Paulo (SP). **Methods:** We assessed biological aging acceleration using six epigenetic clocks based on DNA methylation (methylock package 1.5.0 for R). Immunophenotyping of NK, T, and B cells associated with senescence, exhaustion, and memory was performed via flow cytometry. Additionally, 27 inflammatory mediators were quantified in plasma using a multiplex assay (Bio-Rad Bio-Plex® Pro Human Cytokine Standard). We also evaluated differences in pollutant emissions between the cities based on data from the Air Quality Platform of the Institute for Energy and Environment (IEMA), and compared hospitalization rates for respiratory, infectious, and parasitic diseases per 100,000 inhabitants, using data from the Department of Informatics of the Brazilian Unified Health System (DATASUS), for the period from 2015 to 2019. **Results:** We observed accelerated epigenetic aging, a higher frequency of senescent and exhausted immune cells, and differences in the production of inflammatory mediators in SP residents compared to those in BH. We also identified city-specific correlations between biological aging acceleration, levels of inflammatory mediators, and immunophenotypic profiles. SP showed a greater number of hospitalizations due to respiratory, infectious, and parasitic diseases along with higher emissions of PM₁₀, PM_{2.5}, CO, and NO₂, while BH exhibited higher emissions of O₃ and SO₂. **Conclusion:** Our findings suggest that environmental factors associated with place of residence may influence the development of distinct immunological signatures related to immunosenescence.

Keywords: immunosenescence;remodeling;immunobiography.

2656 - Category: IMMUNOREGULATION (IR)

Role of regulatory T cells in the long lasting effects of oral tolerance induction

DA CONCEIÇÃO, G.R.; NÓBREGA, I.B.C.; ALMEIDA, M.; PINTO, C.H.R.; TORRES, L.; DE OLIVEIRA, M.F.A.; FARIA, A.M.C.. UFMG, BELO HORIZONTE - MG - BRASIL.

The constant exposure of the intestinal mucosa to orally delivered antigens results in a state of suppression of the specific inflammatory response both locally and systemically. This phenomenon, called oral tolerance, relies on the action of regulatory T cells (Tregs) generated in the gut mucosa. A single gavage of antigen is able to suppress immune responses to immunization with adjuvants for up to 3 months after the feeding. However, once established upon immunization, oral tolerance can persist in mice for more than one year. Previous studies from our group showed that the continuous feeding of the antigen for 24 hours is more efficient than single or multiple gavage feedings in suppressing inflammatory diseases, through the Th1 diminished response, the augmented production of TGF- β , IL-10 and CD4+ regulatory T cells. This regimen of feeding is able to maintain the suppressive effects of oral tolerance for a longer period of time. The mechanisms involved in oral tolerance maintenance are still unknown. Thus, the aim of this study is to investigate the immunological mechanisms at place and role of regulatory T cells in maintaining the immunoregulatory effects of oral tolerance over a long period of time. Our study was approved by the Ethics Committee of UFMG (CEUA protocol 263/2024). C57BL/6 mice were orally treated with 20mg OVA by single gavage or with 4mg/mL OVA solution by 24 hours of continuous feeding and immunized with 10 μ g OVA + 3mg Al(OH)₃ i.p 7 or 90 days after the oral treatment. Our preliminary data showed that mice treated with OVA by continuous feeding had a reduction in anti-OVA IgG1 antibodies when immunized 7 and 90 days after oral treatment. Furthermore, these mice presented higher frequencies of CD4+FOXP3+ T cells and FOXP3- ROR γ T+ CD4+ T cells in their spleen and mesenteric lymph nodes, respectively, than control mice. Additional analyses are currently being carried out and data will be presented at the congress.

Keywords: Oral Tolerance;Regulatory T cells;FOXP3.

2665 - Category: IMMUNOREGULATION (IR)

The role of IL-33 in the intestinal alterations associated with obesity.

DE PINHO, A.M.V.F.; DE OLIVEIRA, M.F.A.; AMARAL, N.J.; DE BARROS, H.L.P.; PEIXOTO, J.M.; DA CONCEIÇÃO, G.R.; FARIA, A.M.C.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

The alarmin interleukin 33 (IL-33) is produced mainly by epithelial and endothelial cells. When secreted, it binds to the transmembrane protein ST2 expressed by adipocytes and several immune system cells. IL-33 is related to Th2-type immune responses and, in the adipose tissue, it is involved in regulating adipogenesis and inflammation. Administration of IL-33 leads to reduction of body weight and downmodulation of inflammation in adipose tissue. The intestinal mucosa is also affected by inflammatory alterations in obesity, but little is known about IL-33's role in this context. Based on this, the aim of the study is to establish ST2-/- BALB/c mice as a model for studying the role of IL-33 in the inflammatory alterations observed in the intestinal mucosa in the context of obesity. For this, we fed wild type (WT) and ST2-/- BALB/c mice AIN93G diet as control (WT AIN93G and ST2-/- AIN93G, respectively) and high sugar butter diet (HSB), which is highly caloric (WT HSB and ST2-/- HSB, respectively). After 12 weeks, we collected mice's blood to quantify glucose, triglycerides and cholesterol, as well as the intestine and adipose tissue for histological and gene expression analysis. We observed a 67.32% increase in total body weight and a 43.90% increase in saponadal adipose tissue in ST2-/- HSB, while the other groups did not have an increase greater than 30% in total weight and 20% in saponadal adipose tissue. The ST2-/- HSB group also had increased cholesterol compared to the WT HSB and ST2-/- AIN93G groups. The ST2-/- HSB group had increased glycemia and triglycerides in relation to WT HSB and a trend towards increased glycemia when compared to ST2-/- AIN93G. We are performing PCR analysis for intestinal epithelial integrity and cytokine genes, as well as histological analysis of both intestine and adipose tissue. Our preliminary data suggests that the proposed model is promising for studying the role of IL-33 in the intestinal alterations in the context of obesity.

Keywords: IL-33;intestinal musoca;obesity.

2673 - Category: IMMUNOREGULATION (IR)

Omega-3-induced immunomodulation on murine intestinal mucosa and in *Yersinia pseudotuberculosis* infection.

GIANNOTTI, B.B.¹; ALMEIDA, M.²; TORRES, L.²; PRAZERES, P.H.D.M.²; GONÇALVES, L.M.³; ROSA, N.P.⁴; DE ASSIS, H.C.⁵; OLIVEIRA, B.D.C.³; DA SILVA, G.W.³; MARTINS, V.D.⁶; GOULART, P.P.G.²; DA FONSECA, D.M.²; MAIOLI, T.U.²; FARIA, A.M.C.². 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS - UFMG, NOVA LIMA - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. NEW YORK UNIVERSITY SCHOOL OF MEDICINE, NEW YORK - ESTADOS UNIDOS DA AMERICA; 5. UNIVERSIDADE DE CAMPINAS, CAMPINAS - SP - BRASIL; 6. CEDARS-SINAI, LOS ANGELES - ESTADOS UNIDOS DA AMERICA.

The intestinal mucosa is extensively exposed to a wide range of antigens, which includes the intestinal microbiota, dietary antigens, and also potential pathogens. Beyond its energetic role, the interaction between dietary factors and the microbiota is directly beneficial for the activation and regulation of the immune system. This immune regulation has not only local effects, but also systemic, which leads to an important path of possibilities to use dietary factors as preventive and therapeutic strategies. In this study, we analyzed the immunomodulatory effects of omega-3 (ω 3) supplementation on the intestinal mucosa of mice. Additionally, we investigated the potential regulation of the inflammatory profile by ω 3 supplementation during *Yersinia pseudotuberculosis* infection. Mice were fed with an ω 3-supplemented diet for three weeks prior and during the infection with *Y. pseudotuberculosis*, which was followed for four weeks. Our flow cytometry experiments indicated that dietary supplementation with ω 3 alters the profile of T lymphocytes in the mesenteric lymph nodes (MLN) by decreasing Th1 and Th17 and also in the small intestine lamina propria by lowering Th2 and Th17, representing an anti-inflammatory modulation. Additionally, the ω 3 supplementation reduced the expression of pro-inflammatory cytokine genes. Our study further reveals that ω 3 treatment has a protective effect against *Y. pseudotuberculosis* infection, preventing weight loss, improving clinical outcomes, and also mitigating neutrophil infiltrate in the MLN of mice. Our findings elucidate the importance of considering ω 3 dietary intervention for immune modulation and promising candidate for managing inflammatory responses associated with intestinal infections.

Financial Support: CAPES, CNPq, FAPEMIG and FAPESP.

Keywords: omega-3;inflammation;Yersinia pseudotuberculosis.

2676 - Category: IMMUNOREGULATION (IR)

LACK OF FOOD AVERSION FAVORS FOOD DESENSITIZATION

DE OLIVEIRA, M.F.A.; ALMEIDA, M.; PINTO, C.H.R.; CAMATTA, G.C.; FERNANDES, R.; MOREIRA JUNIOR, R.E.; OLIVEIRA, M.S.; MARTINS, V.D.; LANGER, R.S.; BARBOSA, Y.M.C.; COELHO, M.M.; DE PINHO, A.M.V.F.; DE OLIVEIRA, J.S.; MACHADO, F.S.; OLÓRTEGUI, C.D.C.; GODARD, A.L.B.; SANTOS, V.R.; MAIOLI, T.U.; CARA, D.C.; FARIA, A.M.C.. UFMG, BELO HORIZONTE - MG - BRASIL.

Ovalbumin-sensitized mice avoid drinking egg white solution, an IgE/mast cell-dependent phenomenon called food aversion, seen as a defense mechanism. However, it is not clear if food aversion happens in other types of food allergy. We tested if peanut allergy associates with food aversion and how it affects allergic immune response. BALB/c and C57BL/6 mice were sensitized to peanut protein extract (PPE) and performed a preference test, in which they could choose to drink water or whole peanut extract (WPE). Despite producing specific IgE and inducing mast cell degranulation, sensitized mice from both strains preferred drinking WPE as much as control groups. PPE gavage to control and sensitized mice did not cause activation of amygdala in the brain, but a higher activation of nucleus accumbens in control groups, showing the hedonic value of peanuts. Interestingly, two weeks of WPE ingestion led to a decrease in *Drd2* and *Gabbr2* mRNA in the striatum and behavioral changes associated with a more active reward system in sensitized, but not control groups. Thus, we asked if lack of aversion would lead to chronic antigen exposure and, eventually, desensitization. We sensitized BALB/c mice to OVA or Ara h 1. For three weeks, half of each group could choose to drink water or the antigenic solution, while the other half could only drink the later. When water was available, egg-allergic mice avoided the antigen and, at the end, showed severe anaphylactic reaction and no decrease in specific IgE, unlike the other group. On the other hand, water did not prevent peanut-allergic mice from chronically exposing themselves to the antigen. In the end, both groups presented a decrease in specific IgE and mild anaphylactic reaction. Thus, despite specific IgE and mast cell activation, mice sensitized to peanuts do not display aversion and choose to chronically drink WPE, which might lead to desensitization. Our results challenge the current view on aversion associated with food allergy.

Keywords: Food allergy;Food aversion;desensitization.

2681 - Category: IMMUNOREGULATION (IR)

Regulatory Macrophages as a Therapy for NLRP3 Inflammasome-Driven Inflammation

DE SOUZA, I.A.; MURTA, G.K.T.; PAIXAO, P.H.D.M.; MEIRA, R.D.O.; JÚNIOR, C.M.Q.; AMARAL, F.A.; GONÇALVES, R.. UFMG, BELO HORIZONTE - MG - BRASIL.

Gouty arthritis is an inflammatory joint disease caused by monosodium urate (MSU) crystal deposition. Macrophages play a dual role in this condition, driving both the onset and resolution of inflammation through their ability to adapt activation profiles according to the tissue microenvironment.

This study aimed to explore the therapeutic potential of regulatory macrophages (Mreg) in experimental gout, focusing on reducing inflammation and preventing joint damage. *In vitro* assays were performed using bone marrow-derived macrophages (BMDMs), and *in vivo* experiments employed murine gout models.

Reprogramming macrophages to a regulatory phenotype decreased IL-12 and increased IL-10 secretion compared to other macrophage subsets. Co-incubation of Mregs with LPS-primed macrophages followed by NLRP3 inflammasome activation significantly reduced IL-1 β release, a key cytokine in gouty inflammation. Similarly, in a murine model of acute peritonitis induced by MSU, Mreg administration lowered IL-1 β levels.

Moreover, intra-articular injection of MSU into mouse tibiofemoral joints triggered neutrophilic inflammation, which was mitigated by Mreg therapy through reduced neutrophil infiltration and CXCL1 production.

These findings support further investigation of Mregs and IL-10 in regulating NLRP3 inflammasome-mediated inflammation and suggest that macrophage phenotype modulation may offer a promising therapeutic strategy for gouty arthritis.

Keywords: Regulatory Macrophage;inflammasome NLRP3; Inflammation.

2684 - Category: IMMUNOREGULATION (IR)

Immunomodulatory Effects of *Lactococcus lactis* on the Gut–Pancreas Axis Prior to Type 1 Diabetes Onset in NOD Mice

AMARAL, N.J.; PINTO, C.H.R.; TORRES, L.; OLIVEIRA, M.D.A.; NÓBREGA, I.B.C.; MOREIRA, F.C.; DE OLIVEIRA, J.S.; MOREIRA JUNIOR, R.E.; GODARD, A.L.B.; AZEVEDO, V.A.D.C.; MAIOLI, T.U.; FARIA, A.M.C.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the destruction of pancreatic beta cells by T lymphocytes, resulting in complete insulin deficiency. In addition to genetic factors, environmental elements such as the gut microbiota may influence disease development. This study investigated the use of the probiotic *Lactococcus lactis* NCDO 2118 to modulate the intestinal immune response and its potential to prevent or slow T1DM progression. NOD (Non-Obese Diabetic) mice, aged 4 weeks, were treated or not with *L. lactis* in M17 medium for 28 days, alternating with water. Major gut microbiota phyla were analyzed by qPCR of fecal DNA, and short-chain fatty acid profiles were determined by liquid chromatography. Secretory IgA (sIgA) was quantified by ELISA, and IgA-bound bacteria (Bac-IgA) were analyzed by flow cytometry. Intestinal permeability was assessed by qRT-PCR of tight junctions and FITC-dextran assay. After treatment, mice were euthanized for histological and flow cytometry analysis. *L. lactis* administration significantly reduced ($p=0.0095$) the frequency and severity of pancreatic insulinitis. No significant differences were observed in food and water intake, blood glucose, body weight, sIgA levels, or intestinal permeability. However, Bac-IgA levels were decreased in the treated group. Intraepithelial, T, and B lymphocytes profiles showed no significant changes. Notably, *L.lactis*-treated mice displayed a lower proportion of tolerogenic dendritic cells in the spleen and distal lymph nodes. An increase in total bacteria and *Firmicutes*, and a reduction in *Proteobacteria*, were observed in the colon of the treated group, along with higher levels of acetate and lactate. In conclusion, treatment with *L. lactis* resulted in reduction of insulinitis in NOD mice and in a more immunomodulatory profile during T1DM development, likely influenced by changes in the gut microbiota and the gut–pancreas axis. Funding: FAPEMIG, CNPq.

Keywords: Type 1 diabetes;NOD mice;Probiotics.

2766 - Category: IMMUNOREGULATION (IR)

Effects of IL-17-carrying *Lactococcus lactis* on high-fat diet-induced obesity in experimental model

BARBOSA, S.C.¹; RODRIGUES, V.F.¹; OLIVEIRA, J.E.¹; PACHECO, T.C.F.¹; MACHADO, M.S.G.¹; MAIOLI, T.U.²; WAISMAN, A.³; SARTORI, D.C.¹. 1. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - SP - BRASIL; 3. UNIVERSITY MEDICAL CENTER OF THE JOHANNES GUTENBERG UNIVERSITY OF MAINZ, MAINZ - ALEMANHA.

Obesity leads to intestinal inflammation, increasing intestinal permeability and dysbiosis, which cause visceral adipose tissue (VAT) inflammation, known as meta-inflammation and metabolic disorders. IL-17 is linked to mucosal immunity and metabolic regulation. This study aimed to examine the effects of recombinant *Lactococcus lactis* (LL), which induces IL-17 expression in a high-fat diet (HFD)-induced obesity model in mice. Female C57BL/6 mice were fed a standard diet (SD) or HFD for 6, 12, or 18 weeks. LL administration started at week 6 of HFD and continued every two days until week 12. Mice receiving LL expressing IL-17 were compared to those with an empty plasmid. At week 12, HFD-fed mice showed increased body weight, VAT weight, adiposity index, fasting hyperglycemia, and intestinal permeability, plus decreased IL-17 and IL-22 in the ileum, and fewer Th17 cells in the iliac lymph node. By week 18, obesity-related parameters were aggravated, but intestinal permeability and Th17 cytokine levels were unchanged. LL expressing IL-17 reduced body weight and fat accumulation, improved glucose metabolism, and lowered leptin levels, but did not affect intestinal permeability. LL delivering IL-17 mitigates obesity-induced immunological and metabolic alterations, highlighting the potential of IL-17-carrying probiotics as a treatment strategy for obesity-related metabolic dysfunctions.

Keywords: metabolic syndrome;mucosal immunology;probiotics.

2835 - Category: IMMUNOREGULATION (IR)

The anti-allergic effect of cationic liposomes with CpG using the OVA model of allergic lung inflammation

SANTOS, M.; PERNAMBUCO FILHO, P.C.D.A.; RUSSO, M.; DANTAS, V.A.N.C.. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Asthma is one of the most prevalent diseases in Brazil and anti-allergic immunotherapies are promising alternatives for its treatment. Aluminum salts (alum) have been used safely in vaccines for more than 70 years, however, its undesired pro-Th2 adjuvant activity constitutes a caveat for alum-based vaccines. Even though, alum has been used in immunotherapy. Among promising candidates, lipid nanoparticles stand out for their ability to encapsulate allergens, preventing recognition by anaphylactic antibodies or anti-allergic molecules, like Toll-Like Receptors (TLRs) agonists. This study aimed to investigate the immunoregulatory effects of different lipid formulations in a murine model of allergic asthma, using ovalbumin (OVA) as antigen, administered by subcutaneous (s.c.) or intranasal (i.n.) routes. Cationic liposomes composed of N-[1-(2,3-Dioleoyloxy)propyl]-NN,N,N-trimethylammonium (DOTAP) and CpG oligonucleotides, a TLR9 agonist, were combined with OVA to evaluate their impact on the immune response in C57BL/6 mice. Sensitizations (s.c. or i.n.) and challenges (i.n.) were conducted at weekly intervals. Twenty-four hours after last challenge, bronchoalveolar lavage (BAL) cell counts were quantified, along with histological scores of peribronchovascular infiltrate and mucus production. Levels of total IgE and OVA-specific IgG1, IgG2c in serum and IgA in BAL were measured. Sensitization to OVA with DOTAP given by s.c. but not i.n. route induced Th2-type immunity and increased total IgE and OVA-specific IgA, similar to alum adjuvant. The addition of CpG to DOTAP reduced eosinophilia, mucus and total IgE while increased OVA-specific IgG2c and IgA. Importantly, intranasal DOTAP/OVA+CpG increased neutrophils and OVA-specific IgA in BAL. These findings suggest that CpG suppresses pro-Th2 responses and acts as an anti-allergic modulator when combined with cationic liposomes, supporting the use of lipid formulations in immunotherapies to fine-tune allergic responses.

Keywords: Cationic liposome;CpG;Administration route.

2840 - Category: IMMUNOREGULATION (IR)

Sexual dimorphism in purinergic signalling and regulatory profile of in vitro activated CD4⁺ T-cells

GALLARDO, Z.M.C.; BERGERO, G.; MAZZOCCO, Y.L.; AOKI, M.C.D.C.; ROSSO, S.D.; AOKI, M.D.P.. UNIVERSIDAD NACIONAL DE CÓRDOBA, CÓRDOBA - ARGENTINA.

Background: Several studies have shown that male patients exhibit increased severity of chronic Chagas cardiomyopathy (CCC), although the reasons for this sex disparity remain unclear. Purinergic signalling shapes the outcome of immune activation through the balance between pro-inflammatory ATP and regulatory adenosine (ADO). Potential sex-related differences in purinergic components remain largely unexplored.

Objectives: This study aimed to evaluate the impact of sex on purinergic system components and the regulatory phenotype of activated CD4⁺ T-cells.

Methods: CD4⁺ T-cells were isolated from the spleens of male and female wild-type (WT) and CD73 knockout mice using a negative selection kit. The expression of purinergic and regulatory components was assessed by spectral flow cytometry at 0, 24, 48, and 72 h after activation with CD3/CD28. Additionally, to assess whether ADO promotes differentiation toward a regulatory phenotype, it was added at the onset of activation and evaluated 72 h later.

Results: At baseline, CD4⁺ T-cells from female mice of both genotypes exhibited higher expression of the ATP receptor P2X7 compared to those from male mice ($p < 0.01$). This difference was also observed at 24 h ($p < 0.05$). However, the pattern reversed at 72 h ($p < 0.001$). CD73 expression in cells from WT female and male mice followed similar dynamics ($p < 0.05$ for all time points). The addition of ADO resulted in significantly higher expression of FOXP3 and IL-10 in cells from male mice compared to females at 72 h ($p < 0.01$).

Conclusions: These results suggest that, at baseline, the female sex may promote an anti-inflammatory environment. However, after activation, females exhibited a more reactive profile, characterized by reduced CD73 expression and lower levels of regulatory markers. Although further studies are needed to elucidate the underlying mechanisms, these findings suggest a stronger early immune response in females, which may influence long-term outcomes such as CCC.

Keywords: Adenosine;CD73;IL-10.

2860 - Category: IMMUNOREGULATION (IR)

THP-1 Derived Macrophages as a Model for Evaluating Immunomodulation by Mesenchymal Stromal Cells in a Three-Dimensional Environment

MARTINS, R.; SUZUKAWA, A.A.; DOMINGUEZ, A.C.; MARCON, B.H.. CARLOS CHAGAS INSTITUTE (FIOCRUZ PR), CURITIBA - PR - BRASIL.

INTRODUCTION: In regenerative medicine, the modulation of macrophage behavior by mesenchymal stromal cells (MSCs) has gained prominence, especially through paracrine mechanisms mediated by the secretome. The interaction between MSCs and macrophages can promote a microenvironment more favorable to tissue regeneration. In this study, we developed a protocol for differentiating THP-1 monocytes into M1 and M2 macrophages. These polarized macrophages were subsequently used to assess the immunomodulatory potential of MSCs cultured on 3D SiOC scaffolds, aiming at their application in bone regeneration. **MATERIALS AND METHODS:** THP-1 cells were differentiated into macrophages by treatment with phorbol 12-myristate 13-acetate (PMA). For induction of the M1 phenotype, LPS and IFN- γ were used; for M2, IL-4 and IL-13. Phenotypic characterization was performed by immunofluorescence and Western blot. Additionally, macrophages were co-cultured with MSCs adhered to 3DSiOC scaffolds. After co-culture, RNA extraction and cDNA synthesis were performed to evaluate the expression of genes related to the production of pro- and anti-inflammatory cytokines by qPCR. **RESULTS:** Western blot and immunofluorescence analyses showed that macrophages induced to the M1 phenotype exhibited increased expression of HLA-DR/MHC-II and iNOS, and decreased CD163 levels, compared to M0 and M2 profiles. Cells induced to the M2 phenotype maintained expression levels similar to M0, indicating that polarization to M2 may not have been effective. In the co-culture experiment, a significant reduction in the expression of TNF- α was observed in M1 macrophages treated with 3DSiOC-MSCs, while in co-cultured M2 macrophages, there was an increase in the expression of TGF- β , both compared to their respective controls. **FINAL CONSIDERATIONS:** The protocol was effective for M1 but not for M2. Co-culture with MSCs on 3DSiOC modulated macrophages, suggesting immunomodulatory potential for regenerative medicine.

Keywords: Macrophage polarization; THP-1; Silicon oxycarbide.

2863 - Category: IMMUNOREGULATION (IR)

Effects of macrophage-specific inhibition of ChREBP in obese mice

TAVONI, T.M.¹; CASTRO, G.²; CASTELUCCI, B.G.¹; ANGELIM, M.K.S.C.³; DA COSTA, W.L.G.¹; SANTOS, W.G.S.¹; VIEIRA, P.M.M.D.M.¹. 1. UNICAMP, CAMPINAS - SP - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL; 3. SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE, SAINT LOUIS - ESTADOS UNIDOS DA AMERICA.

Obesity is one of the most prevalent disease with major public health concerns. A chronic low-grade inflammatory state in obesity is exacerbated by the increased presence of pro-inflammatory macrophages. Several factors contribute to macrophage polarization, including the cellular microenvironment and substrate availability, which are key determinants of macrophage phenotype. ChREBP (carbohydrate response element binding protein) leads to reduced inflammatory response and apoptosis by modulating the redox state in LPS-activated macrophages. However, ChREBP-dependent metabolic reprogramming of adipose tissue-resident macrophages remains unclear. We observed that mice with specific deletion of ChREBP in myeloid cells (ChREBP^{-/-}) submitted to a high-fat diet (HFD) for 12 weeks showed lower body fat composition and higher lean mass compared to ChREBP^{+/+} animals. ChREBP^{-/-} HFD animals also exhibited lower gonadal white adipose tissue (gWAT) and subcutaneous white adipose tissue (scWAT) than ChREBP^{+/+} animals. In histological sections of gWAT from ChREBP^{-/-} HFD animals, we observed a reduction in adipocyte diameter compared to ChREBP^{+/+} HFD animals. Additionally, ChREBP^{-/-} HFD-fed animals were more glucose-tolerant and insulin-sensitive. Exploratory analysis included pro-inflammatory and reparative markers in the perigonadal adipose tissue. In comparison to ChREBP^{+/+} animals, even when given a control diet (CD) or HFD, ChREBP^{-/-} animals showed a tendency toward decreased gene expression of IL-6, TNF α , and IL-1 β and a tendency toward increased expression of IL-10 and MGL1/2. In summary, our findings suggest that ChREBP may reprogram adipose tissue-resident macrophages to a pro-inflammatory phenotype. Additionally, ChREBP-dependent metabolic reprogramming of adipose tissue-resident macrophages may be associated to insulin resistance and greater adipose mass in animals fed a HFD.

Keywords: ChREBP; macrophage; immunometabolism.

2904 - Category: IMMUNOREGULATION (IR)

Axl receptor signaling modulates bone loss and oral inflammation in experimental periodontitis

DA COSTA, A.L.A.; NASCIMENTO, J.G.; DE LIMA, J.V.C.S.; DE SÁ, M.C.M.D.M.A.; SOARES, M.A.; DA CRUZ, L.D.O.; FERREIRA, J.R.M.; FILARDY, A.D.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Periodontitis is an infecto-inflammatory condition that leads to oral dysbiosis, exaggerated inflammation, increased apoptosis, and alveolar bone loss (ABL). The clearance of apoptotic cells, termed efferocytosis, is a crucial regulatory mechanism in controlling mucosal inflammation, including the oral cavity. The TAM (Tyro-3, Axl and MerTk) family are tyrosine kinase receptors that indirectly recognize apoptotic cells; efferocytosis mediated by the Axl also regulates TLR and proinflammatory pathways, highlighting its important role in modulating mucosal inflammation. Here, we investigated the role of the Axl receptor in ABL and oral mucosal inflammation during murine experimental periodontitis. C57BL/6J Wild-type (WT) and Axl knockout (KO) mice were submitted to ligature-induced experimental periodontitis (Perio) for 14 days; with an additional resolution experiment with 7 days with ligature followed by 7 days without ligature. The mandibles, gingiva, and cervical lymph nodes were harvested for further analysis.

ABL was less pronounced in Axl KO mice compared to the WT Perio group. Consistent with this, levels of myeloperoxidase, TNF, and IL-1 β in the gingiva during periodontitis were significantly lower in Axl KO mice compared to WT mice. In the cervical lymph nodes, we observed an increased number of ROR γ T+ cells in response to periodontitis in both WT and Axl KO mice. Conversely, lower numbers of FoxP3+ regulatory T cells were found in Axl KO mice compared to WT mice, suggesting that Axl regulates the Treg population among the mucosal sites. Upon the ligature removal experiment, Axl KO animals had a lower ABL recovery compared to WT, which means that Axl participates in the regulation of bone inflammation resolution. Our data suggests that Axl signaling plays a critical role in inducing bone loss and inflammatory cytokine production in periodontitis and may represent a potential target for therapeutic intervention in this pathology.

Keywords: periodontitis;Axl receptor;oral mucosa.

2909 - Category: IMMUNOREGULATION (IR)

Immunomodulatory Effects of Incretin Receptor Stimulation on Intestinal Macrophages in Experimental Colitis

QUADROS-PEREIRA, L.¹; NERY-NETO, J.A.D.O.¹; YARIWAKE, V.Y.¹; ALVES, M.S.²; CAMARA, N.O.S.¹; ANDRADE-OLIVEIRA, V.². 1. USP, SÃO PAULO - SP - BRASIL; 2. UFABC, SANTO ANDRÉ - SP - BRASIL.

Introduction: Ulcerative colitis (UC) affects the colon and rectum and is marked by epithelial damage, microbiota dysbiosis, and immune dysregulation. Macrophages (M ϕ) play a central role in regulating immune responses and maintaining intestinal homeostasis. In UC, they predominantly exhibit an M1-like, pro-inflammatory phenotype. In contrast, an increase in M2-like M ϕ is suggested to lead to a reduction in disease symptoms. Enteroendocrine cells are specialized endocrine cells that respond to luminal nutrients by secreting peptide hormones. Among these hormones, GIP (gastric inhibitory polypeptide) and GLP-1 (glucagon-like peptide-1) stand out as incretins, recognized for their insulinotropic effects. Sitagliptin is a drug that inhibits dipeptidyl peptidase-4, the enzyme that cleaves incretins, thereby increasing their bioavailability. In addition, sitagliptin has demonstrated anti-inflammatory effects in both in vitro and in vivo models across various inflammatory contexts. Objectives: Demonstrate that experimental UC is attenuated through the polarization of intestinal M ϕ toward the M2 phenotype, mediated by increased incretin bioavailability via sitagliptin treatment. Methodology: C57BL/6, Pax-6-Villin-1-cre, Neurogenin-3-RosadTomato and CX3CR1d2 mice will be subjected to seven days of 2% dextran sulfate sodium in drinking water and treated with sitagliptin daily via oral gavage. The Disease Activity Index will be determined based on weight loss, stool consistency, and rectal bleeding. Results: We are currently testing sitagliptin treatment during UC and we will evaluate M ϕ profile during UC. Conclusions: These results reveal whether GLP-1R and GIPR activation will have any impact on UC development and whether sitagliptin attenuates inflammation during UC by increasing the M2 phenotype. Funding: FAPESP (2024/09768-3; 2019/14755-0). Keywords: Intestinal macrophages;colitis;incretins.

2919 - Category: IMMUNOREGULATION (IR)

Eosinophils Activate Macrophages via Paracrine Mechanism: Contribution of Leptin Stimulation

DA SILVA, J.S.; TASMO, N.R.A.; NEVES, L.D.O.F.; CANETTI, C.D.A.; DE MELO, C.B.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Eosinophils express the functional receptor for leptin and control adipose tissue homeostasis, partly due to their ability to modulate macrophages. We have previously demonstrated that co-cultured macrophages and eosinophils mutually affect each other when stimulated by leptin. For example, when in contact with eosinophils, macrophages exhibit increased numbers of cytoplasmic lipid droplets (LDs) and cysLTs synthesis in response to leptin (50 nM; 2 h). The aim here was to characterize whether eosinophil-driven effect is dependent on paracrine activity. To this end, we utilized bone marrow-derived eosinophils (SCF/FLT3 plus IL-5) and peritoneal macrophages from naive BALB/c mice. Instead of co-cultures, we used supernatants from non- (sEos) or leptin-stimulated (sEosLep; for 1 h) eosinophils to activate macrophages (for 2 h). Formation of LDs (osmium-stained cells) and production of cysLTs (ELISA) were analysed in macrophages.

Our results showed that sEosLep increased LD biogenesis and LTC₄ synthesis by macrophages when compared with sEos (n=5; p<0.05). Given that leptin is known to induce the secretion of PGD₂ and CCL5 (RANTES) by eosinophils, the following strategies used to study these mediators in eosinophil/macrophage cross-talking were: (i) inhibition of eosinophil PGD₂ synthesis by HQL-79 (10 µM); (ii) inhibition of G_i protein-coupled receptors in macrophages by pertussis toxin (100 ng/mL); (iii) blockade of RANTES receptors by metRANTES; and (iv) direct induction of LD biogenesis in macrophages stimulated by exogenous PGD₂ (25 nM) or CCL5 (100 ng/mL). While HQL-79 showed no significant effect (n=3), both pertussis toxin (n=2) and metRANTES (n=3) inhibited the formation of new LDs stimulated by sEosLep, and only the direct stimulation with CCL5 (n=3; p<0.05) triggered LD biogenesis in macrophages. Our data indicate that eosinophil/macrophage interaction is partly due to a paracrine activity dependent on soluble mediators derived from eosinophils, including CCL5.

Keywords: Inflammation ;Leukocytes ;Adipose tissue .

2933 - Category: IMMUNOREGULATION (IR)

Consequences of Oral Exposure to Novel Foods in the Experimental Colitis Model

VIDAL, J.L.L.; FERNANDES, B.V.R.; MARMELLO, B.O.; TEIXEIRA, G.A.P.B.. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL.

Introduction. Previous studies have indicated that newly introduced food antigens in animal models with inflammation in the small intestine are associated with the induction of food allergies. Additionally, it is well established that colitis in murine models triggers systemic inflammation. This study aims to assess the impact of colitis-induced inflammation on the development of food allergies and its influence on behavioral changes. Understanding the pathophysiology of these conditions is crucial for effective prevention and the development of new treatments. **Objectives:** Evaluate both the behavioral and physiological effects of the introduction of new food antigens in the context of Dextran Sulfate Sodium (DSS)-induced colitis. **Methods:** animals will be divided in two groups. Group A will receive a 2.5% DSS/water solution *ad libitum* for seven days, while Group B will be given pure water. Following this, subgroups A1 and B1 will be introduced to peanuts in their natural state for 15 days, while subgroups A2 and B2 will continue to eat the standard mouse chow. All groups will be submitted to a peanut-containing challenge diet. Behavioral assessments will be conducted before and after DSS treatment. Finally, blood samples will be collected to titrate anti-peanut IgG and IgE antibodies using ELISA after each intervention, and post-euthanasia, intestinal segments will be harvested for histopathological analysis. **Expected Results:** It is anticipated that high IL-1 and IL-6 expression in the colitis model will trigger an allergic reaction to the newly introduced food and contribute to the onset of depressive and anxious behaviors.

Keywords: food allergy;ulcerative colitis ;stress.

2987 - Category: IMMUNOREGULATION (IR)

The expression of regulatory T cell markers in the adipose tissue of aging mice is modified by high-intensity interval training.

COSTA, M.L.B.; FIGUEIREDO, V.G.; ROCHA, L.E.B.; GARCIA, B.C.C.; SILVA, G.; OTTONE, V.D.O.; COSTA, J.S.R.; RODRIGUES, A.C.D.O.; PEREIRA, A.N.A.; DUARTE, A.L.D.S.; SCHETINO, M.A.A.; BROZINGA, P.F.A.; PIMENTA, A.L.B.; REIS, A.P.G.; QUEIROZ, W.D.M.; SOUZA, M.G.D.S.; SANTOS, J.D.; PEIXOTO, M.F.D.; VIEIRA, E.R.. UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI, DIAMANTINA - MG - BRASIL.

Aging induces significant alterations in the immunometabolic profile of visceral adipose tissue, which are closely associated with systemic metabolic dysfunctions, particularly glucose intolerance and insulin resistance. Given that exercise is a well-established strategy for restoring and maintaining metabolic health and the role of adipose tissue resident regulatory T cells (VAT Treg), this study aimed to investigate the effects of high-intensity interval training (HIIT) on the expression of VAT Treg markers in the visceral adipose tissue of aged mice. Male C57Bl/6 mice (n = 11; 12 months old) were subjected to 16 weeks of HIIT (3 sessions/week, 15 min/session, at 85–100% of maximum running velocity on a treadmill). Morphology of epididymal and retroperitoneal adipose tissues, as well as VAT Treg gene expression (RT-qPCR), were evaluated. Ethical approval for the study was obtained (CEUA UFVJM 5557020623). Data from trained aged mice were compared to those of untrained aged (n = 11) and young animals (n = 8; 4 months old). Compared to young untrained mice, aged untrained mice exhibited reduced expression of VAT Treg markers, FOXP3, PPARG, and IL10, in epididymal but not in retroperitoneal adipose tissue. In the retroperitoneal depot, IL33 expression showed a non-significant trend toward reduction in aged mice compared to young controls (p = 0.08; one-way ANOVA, Holm-Šidák post hoc test). Interestingly, HIIT reversed the age-related downregulation of PPARG and IL10 in adipose tissue. However, the effects of HIIT on FOXP3 and IL33 expression did not reach statistical significance (p = 0.07 and p = 0.08, respectively; one-way ANOVA, Holm-Šidák post hoc test). These findings indicate that aging is associated with reduced expression of VAT Treg-related genes in epididymal adipose tissue, and that this decline can be partially mitigated by exercise training. The authors thank's the Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) for the support.

Keywords: Visceral adipose tissue; ageing; regulatory T cell;

3067 - Category: IMMUNOREGULATION (IR)

Multi-Compartment Profiling by Flow Cytometry and Single-Cell RNA-Sequencing Defines Tregs Alterations in Crohn's Disease

LUDWIG, R.G.¹; MOREIRA, F.C.²; DE CASTRO, M.M.¹; LIMA, G.D.M.³; GONÇALVES, L.M.³; NIRELLO, V.D.¹; TORRES, L.²; GENARO, L.M.¹; PASCOAL, L.B.¹; PEREIRA, L.G.G.¹; AYRIZONO, M.D.L.S.¹; DA FONSECA, D.M.³; LEAL, R.F.¹; VINOLO, M.A.R.¹; FARIA, A.M.C.⁴. 1. UNICAMP, CAMPINAS - SP - BRASIL; 2. UFMG, BELO HORIZONTE - MG - BRASIL; 3. USP, SÃO PAULO - SP - BRASIL; 4. UFMG, BELO HORIZONTE - SP - BRASIL.

Introduction: Disruptions in intestinal immune homeostasis are a hallmark of inflammatory bowel diseases (IBD), such as Crohn's disease (CD). Regulatory T cells (Tregs) are essential for maintaining immune tolerance in the gut, and their dysfunction contributes to CD pathogenesis. Chronic intestinal inflammation-driven remodeling of mesenteric tissues alters lymphatic structures and stromal environments, resulting in a distinctive inflammatory profile. This process may impair immune crosstalk between the gut and mesenteric lymph nodes, compromising mucosal Treg function and increasing susceptibility to inflammation. Although Tregs play a key role in IBD, their compartment-specific functions and gene expression across mesenteric and peripheral sites remain incompletely defined. This study aims to characterize Treg profiles across anatomical compartments using single-cell RNA sequencing (scRNA-seq) and flow cytometry. **Methods:** Single-cell suspensions were prepared from mesenteric adipose tissue (MAT), mesenteric lymph nodes (MLN), and peripheral blood mononuclear cells (PBMC) from individuals with or without CD and analyzed by flow cytometry. Cells were enriched for CD4⁺CD25⁺ through magnetic bead separation and subjected to scRNA-seq using the 10x Genomics Chromium Single Cell 3' Gene Expression platform. Ethical approval was obtained. **Results:** The analysis revealed changes in Treg proportions across tissues and conditions. In CD, the naïve subset of Tregs was reduced in MAT. In PBMC, both naïve and non-naïve Tregs were reduced in the CD group, while increased in MLN tissue. As the study is ongoing, data updates will follow in the poster. **Conclusion:** Analysis suggests spatial redistribution and altered differentiation of Tregs in CD. Naïve Treg redistribution may reflect Treg local and systemic regulatory failure, with impaired transition to effector states. These insights offer a basis for future therapies targeting compartmental immune dysregulation in CD.

Keywords: Crohn's disease (CD); Regulatory T cells (Tregs); Single-cell RNA sequencing.

3081 - Category: IMMUNOREGULATION (IR)

Extracts of *Miconia albicans* (Canela-de-Velho) reduce inflammatory mediators and modulate NLRP3 inflammasome activation in stimulated macrophages

REIS, M.O.; GUEDES, M.C.M.R.; DE OLIVEIRA, L.G.; ALMEIDA, A.P.G.; MACHADO, L.F.; BOLOTARI, M.; PEREIRA, L.G.L.; SEGHEO, J.A.; FABRI, R.L.; MACEDO, G.C.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Introduction: *Miconia albicans* (Melastomataceae), commonly known as “Canela de Velho” is widely used in traditional medicine for the treatment of arthritis, arthrosis, and inflammatory conditions. Despite its popular use, scientific evidence supporting its rational application remains scarce. Therefore, considering the need for new anti-inflammatory therapies and the importance of validating medicinal plants, this study aimed to evaluate the anti-inflammatory potential of aqueous and ethanolic extracts from *M. albicans* leaves. **Methods and Results:** Firstly, the extracts cytotoxicity was assessed by MTT assay in murine macrophage (J774A.1 and RAW264.7) and fibroblast (L929) cell lines. Subsequently, the production of inflammatory cytokines (ELISA) and nitric oxide (NO – Griess reaction) was evaluated in J774A.1 cells stimulated with LPS or LPS + IFN- γ and treated with the extracts. Immunomodulatory activity was investigated in LPS-stimulated cells through CD80 and CD40 expression by flow cytometry. Additionally, the impact of the extracts on the NLRP3 inflammasome pathway was assessed through IL-1 β production and caspase-1 activation (flow cytometry) in LPS + Nigericin stimulated cells. The extracts did not show significant cytotoxicity at the tested concentrations (from 12.5 to 100 μ g/mL), in accordance with ISO10993-5:2009 guidelines. In addition, both extracts reduced IL-12, IL-6, and NO production, and inhibited CD80 and CD40 expression in stimulated J774A.1 cells. Furthermore, the extracts modulated the inflammasome pathway, decreasing IL-1 β production and caspase-1 activation. **Conclusion:** The results demonstrate that *M. albicans* extracts exhibit significant anti-inflammatory and immunomodulatory potential. Further studies are underway to better understand the mechanisms involved and support its therapeutic application. **Financial support:** FAPEMIG (RED - 00067-23 ; RED - 00096-22 ; APQ - 02177-22).

Keywords: Immunomodulation; *Miconia albicans*; Inflammasome.

3097 - Category: IMMUNOREGULATION (IR)

Ferroportin regulates regulatory T cell differentiation and immune homeostasis

LEANDRO, M.D.O.; DA SILVA, E.P.D.; BARBOSA, G.A.D.C.; DAMASCENO, L.E.A.; SEVERINO, M.B.; DE MELO, B.M.S.; CUNHA, T.M.; CUNHA, F.Q.; ALVES-FILHO, J.C.. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Regulatory T cells (T_{regs}) are a subset of CD4⁺ T lymphocytes essential in maintaining immune homeostasis and preventing of excessive immune response. Iron uptake has emerged as a key factor in sustaining T_{reg} function and stability. Ferroportin (FPN), also known as SLC40A1, is an iron exporter that regulates intracellular iron homeostasis by controlling iron efflux. However, its specific role in T_{reg} biology remains unclear. This study investigates the role of FPN in T_{reg} differentiation, function, and its impact on immune regulation and autoimmunity. To explore the involvement of iron-handling pathways in T_{reg} differentiation, naïve CD4⁺ T cells from C57BL/6 mice were cultured under T_{reg}-polarizing conditions. Gene expression analysis revealed a progressive downregulation of *Fpn*, accompanied by an upregulation of *Tfrc* (transferrin receptor 1), a key membrane protein responsible for iron uptake. These changes suggest a transcriptional program favoring intracellular iron retention during iT_{reg} differentiation. To investigate the functional relevance of FPN in T cells, we employed CD4⁺ T cell-specific FPN-deficient mice (CD4^{Cre}FPN^{fl/fl}). *In vitro*, FPN-deficient CD4⁺ T cells exhibited enhanced iT_{reg} cell differentiation, increased expression of immunosuppressive markers, and elevated metabolic activity. *In vivo*, FPN deletion in Foxp3⁺ cells (Foxp3^{YFP-Cre}FPN^{fl/fl}) led to reduced clinical severity and inflammation in the experimental autoimmune encephalomyelitis (EAE) model. Together, these findings demonstrate that ferroportin plays a critical role in the differentiation and function of regulatory T cells, highlighting a novel connection between iron-handling pathways and the maintenance of immune homeostasis.

Keywords: Regulatory T Cell; Ferroportin; Iron metabolism.

3104 - Category: IMMUNOREGULATION (IR)

ROLE OF THE GPR109 RECEPTOR IN THE FOLLICULAR REGULATORY T LYMPHOCYTE GENERATION IN THE GUT AND PROTECTION AGAINST TO TYPE 1 DIABETES IN MURINE MODEL

PARDIM, M.F.; OLIVEIRA, J.E.; NAKAMA, R.P.; AVELINO, S.E.P.; ALVES, B.S.D.L.; RIOS, W.M.; SARTORI, D.C..
UNIVERSITY OF SAO PAULO, RIBEIRAO PRETO - SP - BRASIL.

The pathogenesis of type 1 diabetes (T1D) has been associated with intestinal dysbiosis, which can increase bacterial translocation and disrupt the gut-pancreas axis. Among the microbial metabolites altered in this context, butyrate has recently been reported as an important modulator of mucosal immunity through GPR109 receptor signaling, promoting the regulatory T cell subset generation in the colon, with follicular regulatory T cells (Tfr) representing a potential target due to their role in limiting autoimmunity against self-antigens. To investigate the contribution of GPR109 to Tfr modulation during T1D, C57BL/6 (WT) and *Gpr109^{-/-}* mice were administrated with daily intraperitoneal streptozotocin (STZ) injections (40 mg/kg) for five days. Our results demonstrate that WT mice subjected to STZ-induced T1D developed significant hyperglycemia from day 10, with 80% incidence of T1D, which was maintained until day 15 when compared to the non-diabetic group. In contrast, we found in *Gpr109^{-/-}* mice worsening of blood glucose levels and elevated disease incidence (100%) compared to diabetic WT mice (80%). In parallel, the flow cytometry analysis revealed a trend toward reduced relative frequency and absolute number of Tfr cells in the cecal lymph nodes (cLN) of *Gpr109^{-/-}* mice compared to diabetic WT mice. Moreover, the mean fluorescence intensity (MFI) of PD-1 inhibitory receptor and CD25 expression on Tfr cells appeared to be reduced in *Gpr109^{-/-}* groups without reaching statistical significance, suggesting a possible reduction in the suppressive capacity of Tfrs in these deficient mice. Our preliminary data suggest an important role for GPR109 in modulating the Tfr induction in the gut and protecting against the STZ-induced T1D in experimental model.

Keywords: type 1 diabetes;follicular regulatory T cells;butyrate.

3132 - Category: IMMUNOREGULATION (IR)

Axl regulates the balance between effector and regulatory T cells in mesenteric lymph nodes under homeostatic conditions

DE LIMA, J.V.C.S.¹; DA COSTA, A.L.A.²; NASCIMENTO, J.G.²; RIBEIRO, J.D.O.²; DE SÁ, M.C.M.D.M.A.²; MENDES, C.M.²; MARINS, J.D.G.²; SANTOS, J.M.²; PIMENTA, A.J.²; FERREIRA, J.R.M.²; PINTO, K.G.²; LEITE, J.A.²; FILARDY, A.D.². 1. UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

T lymphocytes (TL) play a crucial role in defense against pathogens and tumors, as well as finely regulating immune responses and tolerance. The intestinal mucosa is continuously exposed to dietary and microbiota antigens, making the balance among T cell populations essential for maintaining homeostasis. Due to the high turnover of intestinal epithelial cells (IECs), efferocytosis becomes vital for the efficient clearance of apoptotic cells and for the release of regulatory cytokines. Additionally, this process provides a pathway for tolerance induction by enabling the presentation of antigens derived from IECs to naïve T cells in mesenteric lymph nodes. Among the receptors involved, Axl, a member of the TAM family, is known to negatively regulate TLR-dependent inflammatory pathways. However, its role in modulating T cell populations remains unclear. To investigate this, we used healthy male 8-10 weeks old C57BL/6 wild-type (WT) and Axl-deficient (*Axl^{-/-}*) mice. Cells from their mLN were harvested, labeled with fluorescent antibodies, and analyzed by flow cytometry to evaluate T cell subsets. In the mLN of *Axl^{-/-}* mice, we observed an increase of Th1 (TCRβ⁺ CD4⁺ Tbet⁺ CD44⁺) and Tc1 (TCRβ⁺ CD8⁺ Tbet⁺ CD44⁺) cells, suggesting an elevated inflammatory tone even in the absence of exogenous stimuli. Additionally, there was a reduction in natural regulatory T cells (CD4⁺ Foxp3⁺ Helios⁺), which are critical for maintaining immune regulation. These data indicate that the absence of Axl promotes a basal pro-inflammatory environment sustained by an imbalance in T cell populations. Functional and proliferation assays are required to further elucidate Axl's role in adaptive immune responses during intestinal homeostasis.

Keywords: T lymphocytes;Axl receptor;Intestinal Mucosa.

3160 - Category: IMMUNOREGULATION (IR)

Investigating the Role of IL-36/IL-38 Axis in Modulating Treg Function in Intestinal Inflammation

SILVA, S.L.S.¹; PACHECO, T.C.F.¹; GARCIA, M.E.M.¹; SANTOS, J.S.²; PARRA, R.S.²; FERES, O.²; FERREIRA, S.D.C.³; RAMOS, A.D.S.¹; CUNHA, F.Q.¹; PERES, R.S.¹. 1. DEPARTMENT OF PHARMACOLOGY, RIBEIRAO PRETO MEDICAL SCHOOL, UNIVERSITY OF SAO PAULO., RIBEIRÃO PRETO - SP - BRASIL; 2. DEPARTMENT OF SURGERY AND ANATOMY, RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO., RIBEIRÃO PRETO - SP - BRASIL; 3. DEPARTMENT OF INTERNAL MEDICINE, RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO., RIBEIRÃO PRETO - SP - BRASIL.

Inflammatory bowel diseases (IBD) involve a complex interplay between pro-inflammatory cytokines and immunoregulatory mechanisms. Among the cytokines implicated in IBD pathogenesis, the IL-36 family (IL-36 α , IL-36 β , and IL-36 γ), has emerged as a key pro-inflammatory mediator, mainly due to its impact on activating epithelial cells, T lymphocytes, and promoting Th17 differentiation. Conversely, IL-38, an antagonist of the IL-36R, has gained attention as a negative regulator of inflammation. However, the effects of these cytokines on regulatory T (Treg) cell responses, which are central to controlling inflammation and maintaining intestinal homeostasis, remain poorly understood. This study investigates the impact of IL-36 and IL-38 on Treg differentiation and function. Murine naive CD4⁺ T cells were isolated and cultured under Treg-polarizing conditions in the presence or absence of recombinant IL-36 γ and/or IL-38. Expression of FOXP3 and of other molecules related to Tregs function were analyzed by flow cytometry and qPCR. We found that IL-36 impaired Treg differentiation, evidenced by reduction in the percentage of FOXP3⁺ cells. In contrast, incubation with IL-38 augmented Treg polarization, supporting its role as a counter-regulatory cytokine. Further work will be performed to characterize IL-36/IL-38 axis in tissue from patients with IBD using the Visium HD spatial transcriptomics platform. In vivo, the therapeutic relevance on targeting IL-36/IL-38 axis will be determined in experimental colitis models using mice with specific deletion of IL-36 in Tregs. Altogether, understanding the role of this axis may provide new insights to better understand the immunological mechanism linked to IBD pathogenesis and may uncover novel therapeutic targets for the treatment of IBD.

Keywords: IL-36/IL-38;Tregs;Intestinal Inflammation;.

3163 - Category: IMMUNOREGULATION (IR)

Discovery of Phage-Derived Antibodies Targeting Ion Channels for the Treatment of Diabetes

FILARDI, E.T.M.; FERNANDES, M.R.; CERNI, F.A.; SANTOS-FILHO, N.A.; PUCCA, M.B.. SCHOOL OF PHARMACEUTICAL SCIENCES, SÃO PAULO STATE UNIVERSITY (UNESP), ARARAQUARA - SP - BRASIL.

Diabetes mellitus is a highly prevalent chronic metabolic disease worldwide, marked by impaired glucose homeostasis and leading to severe autoimmune and degenerative complications. Type 1 diabetes is caused by autoimmune destruction of pancreatic β cells, while type 2 involves insulin secretion defects and metabolic dysregulation. Monoclonal antibody selection via phage display has emerged as a powerful strategy for developing targeted therapies against key molecules involved in these diseases. This study aimed to select phage-derived antibodies specific to Kv1.3 and Kv2.1 ion channels, located on the plasma membrane of T lymphocytes and pancreatic β cells, respectively. Kv1.3 is overexpressed in autoreactive T cells contributing to type 1 diabetes pathogenesis, whereas Kv2.1 is essential for insulin secretion by β cells.

Peptides corresponding to these targets were synthesized by Solid-Phase Peptide Synthesis (SPPS) using the Fmoc strategy and Rink Amide resin. Coupling reactions were performed with DIC and HOBt under controlled conditions to ensure high efficiency. After synthesis, peptides were cleaved using a TFA-based cocktail and purified by preparative HPLC. Purity and identity were confirmed by analytical HPLC and mass spectrometry.

A biopanning approach using streptavidin-coated magnetic beads (Dynabeads®) conjugated to biotinylated peptides allowed for oriented immobilization of Kv1.3 and Kv2.1, preserving their structural integrity. After three rounds of selection and phage amplification, we observed significant enrichment of high-affinity clones. The binding of selected antibodies to the Kv1.3 and Kv2.1 peptides was assessed via bio-layer interferometry (BLI) using the Octet system (Sartorius). This strategy highlights the potential of phage display for generating specific antibody-based candidates for immunometabolic modulation in type 1 and type 2 diabetes, with translational clinical relevance.

Keywords: Phage display;Diabetes mellitus;Immunomodulation.

3168 - Category: IMMUNOREGULATION (IR)

Evaluation of the Immunomodulatory Effects of Daidzein in a DSS-Induced Murine Model of Colitis

PACHECO, T.C.F.¹; SILVA, S.L.S.¹; RAMOS, A.D.S.¹; GARCIA, M.E.M.¹; SANTOS, J.S.²; PARRA, R.S.²; FERREIRA, S.D.C.³; FERES, O.²; CUNHA, F.Q.⁴; PERES, R.S.¹. 1. DEPARTMENT OF PHARMACOLOGY, RIBEIRAO PRETO MEDICAL SCHOOL, UNIVERSITY OF SAO PAULO., RIBEIRÃO PRETO - SP - BRASIL; 2. DEPARTMENT OF SURGERY AND ANATOMY, RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO. RIBEIRÃO PRETO, SÃO PAULO, BRAZIL., RIBEIRÃO PRETO - SP - BRASIL; 3. DEPARTMENT OF INTERNAL MEDICINE, RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO. RIBEIRÃO PRETO, SÃO PAULO, BRAZIL., RIBEIRÃO PRETO - SP - BRASIL; 4. INFLAMMATION AND PAIN LABORATORY, DEPARTMENT OF PHARMACOLOGY, RIBEIRAO PRETO MEDICAL SCHOOL, UNIVERSITY OF SAO PAULO, RIBEIRAO PRETO, SAO PAULO, BRAZIL., RIBEIRÃO PRETO - SP - BRASIL.

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are characterized by chronic and relapsing inflammation of the gastrointestinal tract. Despite therapeutic advancements, effective treatment remains challenging due to an incomplete understanding of the underlying molecular and cellular mechanisms. This study evaluated the immunomodulatory effects of daidzein (DAID), a dietary isoflavone with known anti-inflammatory and antioxidant properties, in a murine model of acute colitis induced by dextran sulfate sodium (DSS). Colitis was induced in C57BL/6 mice by administering 2.5% DSS in drinking water for six days, followed by a two-day recovery period with filtered water. DAID was administered orally at 30 mg/kg/day throughout the eight-day protocol. Disease severity was assessed through weight loss, colon length, histopathological scoring, colonic cytokine gene expression, and flow cytometric analysis of the lamina propria leukocytes (LPLs). DAID treatment significantly attenuated weight loss but did not prevent DSS-induced colon shortening. Notably, DAID-treated mice exhibited enhanced colonic expression of *Il10* and *Pirb*, supporting its anti-inflammatory and immunomodulatory effects. Histological analysis exhibited preservation of mucosal architecture and a reduction of the inflammatory cell infiltration in DAID-treated mice. Flow cytometry analysis revealed increased frequencies of mucosal regulatory T cells (Tregs) in both DSS-exposed groups compared to steady-state controls, with no significant differences between DAID-treated and untreated colitic mice. Collectively, DAID alleviated key features of DSS-induced colitis and promoted anti-inflammatory gene expression, highlighting its potential as an adjuvant therapeutic strategy for intestinal inflammation.

Keywords: Daidzein;Colitis;Regulatory T cells.

3180 - Category: IMMUNOREGULATION (IR)

Critical Role of IL-22 Cytokine in Modulating Type 1 Diabetes through Akkermansia muciniphila Supplementation in Mice

ALVES, B.S.D.L.¹; RODRIGUES, V.F.¹; BARBOSA, S.C.¹; PEREIRA, J.A.¹; PACHECO, T.C.F.¹; OLIVEIRA, J.E.¹; AVELINO, S.E.P.¹; PARDIM, M.F.¹; RIOS, W.M.¹; NAKAMA, R.P.¹; SARTORI, D.C.². 1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, RIBEIRAO PRETO - SP - BRASIL.

Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by a failure in immune self-tolerance, resulting in the activation of autoreactive T lymphocytes and the progressive destruction of pancreatic β -cells, ultimately leading to severe insulin deficiency. Furthermore dysbiosis, driven by both extrinsic and intrinsic factors such as diet, infections, toxins, and genetic predisposition, has been linked to inflammatory and autoimmune diseases, including immune and metabolic disorders like T1D. This scenario highlights the importance of exploring innovative therapeutic approaches to minimize disease progression and its microvascular and macrovascular complications, which directly impact the quality and life expectancy of diabetic individuals. In this study, we evaluated the effects of supplementation with the *Akkermansia muciniphila* probiotic in a streptozotocin-induced murine model of T1D. Initially, we observed a positive correlation between IL-22 levels and glycemic improvement suggests a functional role for this cytokine in the protective response. Second, our results demonstrated that mice supplemented with *A. muciniphila* showed partial protection against T1D development, significantly lower blood glucose levels, and increased IL-22 expression in the colon and ileum compared to the diabetic group. Later, we also found increased expression of epithelial junction proteins (occludin and ZO-1) and mucins, indicating improved intestinal barrier integrity in supplemented mice compared to diabetic mice. Additionally, flow cytometry analysis of pancreatic lymph nodes (pLN) revealed a significant increase in the absolute number of regulatory T cells (Tregs, CD3⁺CD4⁺Foxp3⁺) in diabetic mice supplemented with *A. muciniphila*. Taken together, these findings demonstrate that *A. muciniphila* supplementation confers IL-22-mediated immunoregulatory effects in the gut-pancreas axis and represents a promising approach for the prevention and management of T1D.

Keywords: Akkermansia muciniphila;Type 1 diabetes;IL-22.

3183 - Category: IMMUNOREGULATION (IR)

Recombinant tropomyosin Lit v 1 for nanoparticle-based oral immunotherapy in a shrimp allergy mouse model

PINTO, N.D.S.¹; ANDRADE, C.M.²; RAMOS, K.D.S.³; LIMA, E.³; ROCHA, V.P.C.³; SOARES, M.B.P.²; BRODSKYN, C.I.². 1. INSTITUTE GONÇALO MONIZ - FIOCRUZ BAHIA, SALVADOR - BA - BRASIL; 2. INSTITUTE GONÇALO MUNIZ- FIOCRUZ BAHIA, SALVADOR - BA - BRASIL; 3. INSTITUTE OF HEALTH TECHNOLOGY SENAI CIMATEC, SALVADOR - BA - BRASIL.

Shrimp allergy is among the most common food allergies and a leading cause of severe food-induced anaphylaxis, often triggered by accidental exposure. Current immunotherapeutic approaches carry significant risks of systemic reactions, highlighting the urgent need for safer alternatives. Nanoparticle-based allergen delivery offers a promising strategy to induce long-lasting oral tolerance by modulating immune responses in the gut. In this study, the Lit v 1 gene (GenBank No. EU410072.1), the major shrimp allergen from *Litopenaeus vannamei*, was synthesized and cloned into the pET28a(+) vector with an N-terminal 6×His tag. The construct was transformed into *Escherichia coli* BL21(DE3), and protein expression was induced using 0.2 mM or 1 mM Isopropyl β-D-1-thiogalactopyranoside IPTG at 18°C or 37°C for 3 hours. Recombinant Lit v 1 was purified from cell lysates using Ni-NTA affinity chromatography and analyzed by SDS-PAGE and Western blotting. Similar expression levels were observed under all tested conditions, yielding soluble protein with high purity and concentration (~2 mg/mL, ~37 kDa). Additionally, poly (lactic-co-glycolic acid) (PLGA) nanoparticles coated with chitosan (5 mg/mL) were synthesized using the solvent evaporation method. Incorporation of 0.2% Tween 20 as a dispersant reduced the polydispersity index, improving particle homogeneity, although the average particle size remained relatively large (>1000 nm). These results demonstrate the feasibility of producing recombinant tropomyosin suitable for nanoparticle encapsulation with favorable physicochemical characteristics. Our findings support the potential of nanoparticle-encapsulated tropomyosin as a novel oral immunotherapy strategy, promoting intestinal immune tolerance and contributing to the development of safer, more effective treatments for food allergies.

Keywords: Shrimp allergy; Recombinant tropomyosin; Immune tolerance.

3197 - Category: IMMUNOREGULATION (IR)

RECOMBINANT PROTEINS FROM *Trichuris trichiura*: IN VITRO TOXICITY AND IN VIVO IMMUNOTHERAPEUTIC POTENTIAL

DE ANDRADE, J.C.D.C.¹; SOUSA, J.E.A.¹; SOUZA, J.L.F.M.²; ORRICO-FERREIRA, C.M.¹; SOUZA, L.M.¹; BELITARDO, E.M.M.D.A.¹; DA SILVA, E.S.¹; PINHEIRO, C.D.S.¹. 1. FEDERAL UNIVERSITY OF BAHIA, SALVADOR - BA - BRASIL; 2. GONÇALO MONIZ INSTITUTE, SALVADOR - BA - BRASIL.

Introduction: Allergic diseases affect millions of people worldwide. Helminths have shown potential in modulating allergic responses in the host. Omics studies conducted by our group identified TtMIF and Ttc4299 proteins as potential modulators of inflammatory responses. **Objective:** To evaluate the cytotoxicity of the recombinant proteins rTtMIF and rTtc4299 from *Trichuris trichiura* in different cell types and rTtc4299 immunomodulatory potential in a murine model of *Blomia tropicalis*-induced allergy. **Methods:** The rTtc4299 protein was expressed in *E. coli* BL21 (DE3) and purified using an ÄKTA Pure 25® system with a HisTrap FF Ni Sepharose column. SDS-PAGE and Western blot confirmed expression and purity. Cytotoxicity was evaluated in THP-1 cells via MTT assay. The immunomodulatory capacity of rTtc4299 was assessed through cytokine quantification in cell supernatants using ELISA. A/J mice were sensitized and challenged with *Blomia tropicalis* extract, then treated subcutaneously with dexamethasone or rTtc4299. Regulatory T cells in splenocytes were analyzed by flow cytometry. **Results:** Stimulation with the proteins did not induce toxicity in THP-1 cells. The rTtc4299 stimuli induced the increased secretion of IL-10, IFN-γ, and IL-1β levels, while reducing TNF levels, indicating its immunomodulatory properties. Total and differential leukocyte counts in the bronchoalveolar lavage fluid of mice were modulated by rTtc4299 treatment. Antibodies (IgG, IgG2a and IgE) and cytokine responses (IL-10, IL-4, IL-5 and IFN-) were also modified in mice treated with rTtc4299. Flow cytometry analyses revealed that rTtc4299 treatment influenced T cell regulatory responses. **Conclusion:** *T. trichiura* recombinant proteins seem to be non-toxic and capable of modulating the Th2 immune response *in vitro* and *in vivo* promoting a mixed immunological profile with regulatory and Th1 cytokine production. However, additional assays are still required to confirm these findings

Keywords: Allergy; immunomodulation; whipworm.

3220 - Category: IMMUNOREGULATION (IR)

Gram-negative bacteria exacerbate colitis through epithelial senescence

NIRELLO, V.D.¹; DE OLIVEIRA, S.¹; DE ASSIS, H.C.¹; VINOLO, M.A.R.¹; BERAZA, N.²; VARGA-WEISZ, P.¹. 1. UNICAMP, CAMPINAS - SP - BRASIL; 2. QUADRAM INSTITUTE, NORWICH - REINO UNIDO.

The composition of the gut microbiota can critically influence the outcome of colitis, either promoting resolution or aggravating epithelial damage and inflammation. While this has been widely attributed to microbial metabolites and immune modulation, less is known about how specific bacteria directly affect epithelial cell fate. Cellular senescence, typically associated with aging, can also be triggered by stressors such as inflammation, DNA damage, or microbial cues. However, the role of stress-induced epithelial senescence in intestinal disease remains poorly understood. Here, we show that gram-negative bacteria trigger epithelial senescence and worsens colitis.

Broad-spectrum antibiotics aggravated DSS-induced colitis in mice, accelerating disease onset and severity while creating a depleted microbiota environment permissive to the overgrowth of gram-negative such as *Enterobacteriaceae* and *Pseudomonadaceae*. Among selective antibiotics, vancomycin—which targets gram-positive bacteria—mimicked the effect of broad-spectrum treatment, while colistin—which targets gram-negatives—did not, supporting a gram-negative-dependent mechanism. Consistently, epithelial senescence was induced by vancomycin treatment, *E. coli* colonization in germ-free mice, and *Salmonella* infection in SPF mice. Senescence markers such as p16 were elevated in these models, as well as in colitic mice and inflamed intestinal tissues from IBD patients, where they correlated positively with inflammation and were enriched near the luminal surface. Single-cell and histological analyses of IBD patient confirmed epithelial senescence and its spatial association with inflamed regions. Importantly, senolytic treatment (ABT-263) reduced epithelial senescence and improved disease outcomes in vivo.

These findings identify stress-induced epithelial senescence as a key mechanism by which gram-negative bacteria disrupt mucosal homeostasis and highlight senescence as a potential therapeutic target in IBD.

Keywords: Epithelial senescence; Gut microbiota; Colitis.

3242 - Category: IMMUNOREGULATION (IR)

Modification of Chimeric Antigen Receptor (CAR) Vector for the Inclusion of Genetic Elements to Modulate T Lymphocyte Exhaustion Phenotype

WERNECK, I.V.T.¹; SOUSA, I.G.¹; STUDART, I.C.¹; ARAGÃO, D.R.¹; DE SOUZA, P.V.D.¹; BONAMINO, M.H.²; MARANHÃO, A.Q.¹; BRIGIDO, M.D.M.¹. 1. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL; 2. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

CAR-T cell immunotherapy, which uses genetically modified T lymphocytes to eliminate tumor cells, faces the challenge of cellular exhaustion induced by inhibitory receptors and continuous exposure to antigens and the immunosuppressive microenvironment. To overcome this, gene regulation strategies using Mirtrons were proposed here. Selected targets include the transcription factor TOX, linked to epigenetic exhaustion signals, and miR-29a, whose enhanced expression may inhibit exhaustion pathways and promote a memory phenotype. To reprogram T cells via mirtron-based genetic elements, CAR-producing vectors were modified to include this intron-like sequences in the CAR transgene. The CAR vector pT4 BcA MSCV'U3 19BBz (FMC63) were modified to enable mirtron delivery in different regions of the coding transgene (5'UTR and CDS). Three siRNAs targeting TOX and the miR-29a were converted to a Mirtron format. Eight experimental vectors and one reporter based on the psiCHECK-2-XNS vector were constructed. Five vectors were used for protocol optimization, CAR expression, and gene regulation analysis. Plasmids were produced in *E. coli*, purified, and transfected into HEK293T cells using Lipofectamine 3000. After 48 hours, a dual-luciferase assay and flow cytometry immunophenotyping were performed. Shifts in the cell population by flow-citometry supported CAR expression on the cell membrane, confirming the correct processing of Mirtrons at varied efficiency. Moreover, Mirtron bearing vectors effectively reduced the Firefly/Renilla ratio in trans, demonstrating the generation of biologically active siRNA. Results suggest proper Mirtron processing and its functionality as post-transcriptional regulators. This approach shows potential to reduce T cell exhaustion and preserve effector and memory functions.

Keywords: Exhaustion; CAR-T; Mirtrons.

3308 - Category: IMMUNOREGULATION (IR)

Study of immunometabolic adaptation mechanisms during experimental sepsis and the development of neurocognitive sequelae

CORDEIRO, A.D.F.; TERRA, E.; DA LUZ, C.S.; PEREIRA, L.M.M.; DOS SANTOS, P.D.M.A.; ALVES, R.F.; TAKEUTI, V.Y.; REIS, P.A.. STATE UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

In 2024, Brazil reported 14,000 sepsis cases, with fatality rates of 21% for sepsis and 53% for septic shock, based on 2023 data. Sepsis is an organ dysfunction from complex host-pathogen interaction, triggering a dysregulated systemic response leading to often fatal hemodynamic, microcirculatory, and metabolic disturbances. The Central Nervous System (CNS) is frequently affected, causing altered mental status during inflammation, though the exact mechanisms for these cognitive changes remain unknown. Our study aims to understand how sepsis's uncontrolled inflammatory response impacts the CNS, causing neuroinflammation and long-term cognitive sequelae. To achieve our objective, we used C57BL/6 mice, mimicking human sepsis via the cecal ligation and puncture (CLP) model, which induces moderate-to-severe polymicrobial sepsis (50% mortality in 24h). Post-CLP (24h), animals showed glycemic dysfunctions, initially hyperglycemia followed by hypoglycemia for up to 72h. Serum ELISA analyses (6h and 24h post-CLP) revealed significant cytokine profile alterations, including increased IL-6, IL-1, TNF- α , MCP-1, and CXCL-1. To assess cognitive damage, the freezing test was performed on days 7 and 15 post-CLP, indicating significant short- and long-term memory deficits ($p < 0.05$). On day 15, the Morris water maze (MWM) demonstrated marked spatial memory impairment ($p < 0.01$). These preliminary results suggest that sepsis-induced systemic inflammation directly impacts the CNS, contributing to cognitive impairment. Our data highlight the importance of further investigating neuroinflammatory mechanisms for understanding and potentially developing interventions for sepsis's neurological sequelae.

Keywords: Sepsis; Central Nervous System; Immunometabolism.

3325 - Category: IMMUNOREGULATION (IR)

The ectonucleotidase CD39 regulates in vitro activation and proliferation of CD4⁺ T cells

MATTOS, G.H.R.¹; DE SOUZA, D.C.D.S.R.¹; MANHÃES, L.D.S.B.¹; FREITAS, R.J.R.X.¹; CODIGNATO, S.G.¹; SANTOS, G.D.A.²; ROBSON, S.C.³; SANTIAGO-CARVALHO, I.⁴; LIMA, M.R.D.¹. 1. DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. CARTER IMMUNOLOGY CENTER, DIVISION OF INFECTIOUS DISEASES AND INTERNATIONAL HEALTH, UNIVERSITY OF VIRGINIA SCHOOL OF MEDICINE, CHARLOTTESVILLE - ESTADOS UNIDOS DA AMERICA; 3. BETH ISRAEL DEACONESS MEDICAL CENTER, BOSTON - ESTADOS UNIDOS DA AMERICA; 4. DEPARTMENT OF IMMUNOLOGY, MAYO CLINIC, PHOENIX - ESTADOS UNIDOS DA AMERICA.

The ectonucleotidase CD39 is expressed by both immune and non-immune cells and cleaves extracellular ATP (eATP) into ADP and AMP, regulating purinergic signaling through P2X receptor activity. Among these, P2RX7 stands out for requiring high eATP concentrations to become activated, typically under pathological conditions. In this context, our group demonstrated that in response to severe tuberculosis (TB), CD39 acts as an essential regulator of TB-infected macrophages in the lung. In another study, we showed that T cell-specific P2RX7 activation in response to TB and influenza (flu) promotes CD4⁺ T cell accumulation in the lung parenchyma, where these cells may act pathogenically by exacerbating inflammation and worsening infection outcomes. Despite these findings, the role of CD39 in CD4⁺ T cell responses during severe pulmonary infections remains unexplored. Here, we aim to investigate CD39's role in CD4⁺ T cell activation and proliferation in vitro. For this, CD4⁺ T cells from WT and CD39-KO mice were isolated by negative selection, cultured with anti-CD3/CD28 and IL-2 for 24h, 48h, and 72h. A significant increase in activated CD39-KO CD44⁺CD4⁺ cells was observed at 48h and 72h compared to WT. However, we detected a lower frequency of CellTrace⁻ CD39-KO CD4⁺ T cells, suggesting reduced proliferation. No significant differences in cell death were observed, as Live/Dead⁺ cell frequencies were similar between groups. Phenotypic analysis using activation and differentiation markers showed a late (48h) increase in CXCR3 and T-bet expression in CD39-KO CD4⁺ T cells. Our previous data showed that eATP signaling induces CXCR3 expression, promoting accumulation of pathogenic T-bet⁺CXCR3⁺CD4⁺ T cells in the lung parenchyma. As CD39 deficiency increases free eATP, it may enhance P2RX7 activation and drive excessive accumulation of these cells, potentially worsening lung tissue damage. Testing this hypothesis in flu in vivo models is part of our next steps.

Keywords: Purinergic Signaling; CD39; Pathogenic T cells.

3358 - Category: IMMUNOREGULATION (IR)

Effect of Mygalin on the inflammatory response induced by the combination of Toll-like receptor agonists

CAVALCANTE, M.D.O.¹; MENDES, E.²; DA SILVA, K.C.D.²; NOGUEIRA, J.N.²; DA SILVA JUNIOR, P.I.²; BORGES, M.M.². 1. UNIVERSIDADE DE SÃO PAULO - USP, SÃO PAULO - SP - BRASIL; 2. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

The simultaneous stimulation of different Toll-like receptors stands out as a strategy to modulate the immune response, due to its enhancement of activation, improving the efficacy of vaccines and anti-infectious and anti-tumor immunotherapy. Mygalin is a synthetic acylpolyamine with microbicidal activity against *E. coli*. It is non-cytotoxic to eukaryotic cells and reducing the inflammatory response. The combination of TLR4 agonists, Monophosphoryl Lipid A (MFLA), and Resiquimod (R848), a TLR7 agonist, has been used as adjuvants to stimulate the innate and adaptive response, with potential for infection control and development of new therapies.

Objective: To analyze in vitro the effect of adding Mygalin on the inflammatory response of RAW 246.7 cells during the combination of agonists of cell surface receptors, TLR4 (MFLA) and intracellular TLR7 (Resiquimod).

Methodology: RAW 264.7 cells were cultured in complete RPMI medium. The cells were pretreated with Mygalin and subsequently activated with agonists MFLA (TLR4) and R848 (TLR7/8) individually or in combination for 20–24 hours. After this period, the supernatant was collected and used for the measurement of immune mediators.

Results: Macrophage activation by MFLA, R848, or a combination of both induced a dose-dependent response, increasing the production of NO, IL-6, IL-12p40, and the chemokine MCP-1. When we analyzed the interference of Mygalin on macrophage activation with the agonists MFLA, R848, and the combination of both, we observed a reduction in the synthesis of NO, IL-6, IL-12p40, and MCP-1.

Conclusions: The data indicate that Mygalin attenuates the production of inflammatory mediators during macrophage activation with the agonists individually or in combination, reducing the immunomodulatory effect of TLR agonists. The reduction of the inflammatory response by Mygalin when combined with TLR agonists may have therapeutic and biotechnological applications for the development of immunomodulatory therapies.

Keywords: Innate immunity; Mygalin; TLR4 and TLR7.

3365 - Category: IMMUNOREGULATION (IR)

Study of the the Modulation of Macrophage Phenotype by PLIN1, SAA1, and Leptin Adipocytes

SACRAMENTO, I.R.F.¹; SILVA, L.F.L.¹; DA COSTA, W.L.G.¹; GAN, F.C.²; SPONTON, C.H.G.²; ANGELIM, M.K.¹; VIEIRA, P.M.M.D.M.¹. 1. LABORATORY OF IMMUNOMETABOLISM, DEPARTMENT OF GENETICS, EVOLUTION AND BIOAGENTS. INSTITUTE OF BIOLOGY, UNIVERSITY OF CAMPINAS, CAMPINAS, SÃO PAULO, BRAZIL., CAMPINAS - SP - BRASIL; 2. DEPARTMENT OF PHISIOLOGY, UNIVERSITY OF SÃO PAULO SCHOOL OF MEDICINE, RIBEIRÃO PRETO, SÃO PAULO, BRAZIL., CAMPINAS - SP - BRASIL.

The low-grade chronic inflammation observed in obesity is closely associated with the interaction between adipocytes and macrophages, contributing to metabolic dysfunction and insulin resistance. Previous studies have shown that, in an obesogenic environment, the genes Plin1 and Saa1 are downregulated, while Leptin is overexpressed in adipose tissue macrophages. In this context, we aim to determine how Plin1, Saa1 and leptin expression in adipocytes impact macrophage phenotype. We generated three genetically modified adipocyte cell lines via CRISPR-Cas9: (i) 3T3 overexpressing Plin1, (ii) 3T3 overexpressing Saa1, and (iii) 3T3 with Leptin deletion. The target proteins were selected based on their relevant roles in lipid metabolism and inflammatory signaling: PLIN1 regulates lipid storage in lipid droplets, SAA1 acts as an acute-phase protein with chemotactic potential, and leptin is a pro-inflammatory adipokine. The genetic modifications were validated by qPCR for Plin1 and Saa1, and by sequencing for Leptin. These cell lines were then differentiated into adipocytes and co-cultured with macrophages where they differentially affect macrophage phenotype. Together, by using this in vitro model we will elucidate how the secretory profile of adipocytes modulate macrophage phenotype and metabolism, thus advancing the understanding of the immunometabolic regulation of macrophages in obesity.

Keywords: Macrophages; Adipocytes; metabolism.

3366 - Category: IMMUNOREGULATION (IR)

Evaluation of the recombinant immunotherapeutics BTH2 and rDer p 2231 in a murine model of allergic co-sensitization

DOS SANTOS, S.L.B.; SOUSA, J.E.A.; SOUZA, L.M.; DE ANDRADE, J.C.D.C.; SANTOS, M.L.; COSTA, M.C.D.S.; CRUZ, P.E.D.O.; SENA, N.C.; FERNANDES, A.M.S.; ORRICO-FERREIRA, C.M.; SILVA1, R.C.; DA SILVA, E.S.; PINHEIRO, C.D.S.. UFBA, SALVADOR - BA - BRASIL.

Abstract: Respiratory allergic diseases such as asthma are a growing public health concern in Brazil. Allergen-specific immunotherapy (AIT) is the only treatment capable of modifying disease progression, but current approaches based on crude extracts present variability and risk of adverse reactions. Our group developed the recombinant hypoallergens BTH2 (*Blomia tropicalis*) and rDer p 2231 (*Dermatophagoides pteronyssinus*), which previously demonstrated therapeutic potential in *in vitro* studies and acute murine models. This study evaluated the combined use of BTH2 and rDer p 2231 in an acute murine model of allergic co-sensitization to *B. tropicalis* and *D. pteronyssinus*. A/J mice were sensitized with mite extracts and treated subcutaneously with the hypoallergens during the challenge phase. Inflammatory and immunological parameters, including airway inflammation, antibody responses, cytokine profiles and lung histopathology, were assessed. Overall, immunotherapy with BTH2 and rDer p 2231 was associated with reduced airway inflammation and modulation of type 2-associated responses, favoring regulatory mechanisms and the induction of protective antibody profiles. These findings support the potential of a combined recombinant allergen-based AIT strategy for co-sensitized patients. This preclinical study represents a relevant step toward the development of a more precise, safe and effective therapeutic approach, with the potential to improve quality of life and reduce the health burden associated with allergic respiratory diseases.

Keywords: allergen-specific immunotherapy; recombinant hypoallergens; co-sensitization.

3370 - Category: IMMUNOREGULATION (IR)

Topical Application of rSm29-Conjugated Gold Nanoparticles in a Gel Formulation Modulates DNCB-Induced Dermatitis in an Experimental Model

CARDOSO, L.S.¹; GOMES, R.D.O.²; SOARES, D.D.M.³; SANTANA, J.B.⁴; DALTRO, S.R.T.⁵; MIRANDA, C.B.S.¹; MACHADO, P.R.⁴; OLIVEIRA, S.C.⁶; CARVALHO, E.M.⁵; CARDOSO, T.M.D.S.⁵. 1. FACULDADE DE FARMÁCIA, UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. FACULDADE DE FARMÁCIA, UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR, BA, BRASIL., SALVADOR - BA - BRASIL; 3. FACULDADE DE FARMÁCIA, UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR, BA, BRASIL, SALVADOR - BA - BRASIL; 4. SERVIÇO DE IMUNOLOGIA, HUPES, UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 5. FIOCRUZ-BA, SALVADOR - BA - BRASIL; 6. LABORATÓRIO DE IMUNOLOGIA DE DOENÇAS INFECCIOSAS-USP, SÃO PAULO, SP, BRASIL, SALVADOR - BA - BRASIL.

Atopic dermatitis is a chronic, inflammatory allergic-based disease associated with different clinical phenotypes that typically begins in early childhood. Studies have shown that helminth infections, or their products, can induce a regulatory immune response capable of modulating diseases marked by excessive immune activation. In this context, helminth-based therapy has emerged as a promising therapeutic strategy for hyperinflammatory diseases, as seen in atopic disorders. The aim of this study was to evaluate the potential of a gel containing the recombinant *Schistosoma mansoni* antigen (rSm29) conjugated to gold nanoparticles as a topical treatment in a murine model of atopic dermatitis induced by dinitrochlorobenzene (DNCB). The experimental protocol, approved by the Animal Ethics Committee (CEUA - Fiocruz-IGM, protocol 11/2023), involved exposure to the inducing agent at different concentrations and time points. Animals were treated with standardized amounts of rSm29+AuNP gel, placebo (AuNP gel), or dexamethasone cream applied to the dorsal skin. At the end of the protocol, the animals were euthanized and biological samples were collected for analysis. The results showed a significant reduction in clinical severity scores, epidermal thickness, and mast cell migration ($p < 0.05$) in comparison to the control and placebo groups. There was also a reduction in mast cell migration in ear biopsies from animals treated for a longer period—an anatomical site distant from the sensitization area and not directly treated ($p < 0.05$). In splenocyte cultures, an increase in the levels of the regulatory cytokine IL-10 was observed following *in vitro* stimulation with rSm29+AuNP, compared to other stimuli ($p < 0.05$). These results suggest that the rSm29+AuNP gel has the potential to be used as a topical immunomodulator in the treatment of atopic dermatitis.

Keywords: Atopic dermatitis; rSm29; Atopic eczema.

3415 - Category: IMMUNOREGULATION (IR)

DDX41 in the Stability and Function of Regulatory T Cells

CASTRO, M.V.D.S.; CHIARI, G.C.S.; PÚBLIO, G.A.; DE MELO, B.M.S.; DE OLIVEIRA, C.V.; BARBOSA, G.A.D.C.; DAMASCENO, L.E.A.; CUNHA, F.Q.; CUNHA, T.M.; ALVES FILHO, J.C.F.. UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Regulatory T cells (Tregs) play a crucial role in preventing excessive immune responses and maintaining immune homeostasis. DDX41, a member of the DEAD-box RNA helicase family, is well known for its involvement in RNA processing and innate immune signaling. However, its role in adaptive immunity remains poorly understood. Through bioinformatic analysis using the STRING database, we identified a potential interaction between DDX41 and the master Treg transcription factor FoxP3, suggesting a previously unrecognized link between this helicase and Treg biology. To explore this, we investigated the role of DDX41 in the differentiation and function of Tregs. Analysis of gene expression datasets from the ThExpress Atlas revealed that *Ddx41* is highly expressed in both natural (nTregs) and induced Tregs (iTregs), suggesting a specific role in these subsets. *In vitro* experiments confirmed that DDX41 mRNA levels increase during early iTreg differentiation compared to naïve CD4⁺ T cells. Moreover, conditional knockout of *Ddx41* in CD4⁺ T cells led to a marked reduction in the CD4⁺FoxP3⁺ population following TGF- β -induced differentiation, indicating that DDX41 is required for proper Treg development. Collectively, these findings uncover a novel function for DDX41 in adaptive immunity, highlighting its involvement in molecular pathways essential for Treg induction and maintenance. DDX41 emerges as a critical regulator of Treg biology and a potential therapeutic target in autoimmune and inflammatory disorders.

Keywords: FoxP3;DDX41;Regulatory T cells.

3438 - Category: IMMUNOREGULATION (IR)

M2 MACROPHAGE POLARIZATION TOWARD A TOLEROGENIC PHENOTYPE AND IL-10 PRODUCTION INDUCED BY *Akkermansia muciniphila*-DERIVED EXTRACELLULAR VESICLES (AmOMV)

NAKAMA, R.P.; RODRIGUES, V.F.; OLIVEIRA, J.E.; ALVES, B.S.D.L.; AVELINO, S.E.P.; RIOS, W.M.; PARDIM, M.F.; ALMEIDA, F.B.D.R.; SARTORI, D.C.. FMRP-USP, RIBEIRÃO PRETO - SP - BRASIL.

There is evidence about an inverse correlation between metainflammation and the abundance of the *Akkermansia muciniphila* probiotic bacterium during the progression of metabolic diseases like type 2 diabetes mellitus (T2D). Outer membrane vesicles (AmOMV) derived from *A. muciniphila* support epithelial barrier integrity and reduce pro-inflammatory cytokines, suggesting their potential to modulate metainflammation. This study therefore aimed to investigate the *in vitro* effects of AmOMV the differentiation and activation of bone marrow-derived macrophages into M2 profile. AmOMV were isolated by ultracentrifugation and characterized by Nanoparticle Tracking Analysis, revealing a mode size of 151.9 $\pm$ 37.2 nm, confirmed by transmission electron microscopy. Bone marrow cells from C57Bl/6 (1 $\times$ 10⁶/mL) were cultured with GM-CSF (20 ng/mL) for 3 days and then stimulated with retinoic acid (RA; 2 μ M) or AmOMV at different concentrations (1 $\times$ 10⁷, 1 $\times$ 10⁸, or 1 $\times$ 10⁹ particles/well). On day 7, cells were collected for analysis of cell viability, biomarkers like CD64 and CD11c by flow cytometry, IL-10 levels in supernatants by ELISA assay, and gene expression (*Adgre1*, *Nos2*, *Arg1*, *Mrc1*) by RT-qPCR (CEUA 1432/2025). The stimulation with AmOMV in the concentration of 1 $\times$ 10⁷ did not affect cell viability compared to controls (medium and RA), while AmOMV in the concentrations of 1 $\times$ 10⁸ and 1 $\times$ 10⁹ resulted in 66.25% and 59.42% cell viability, respectively. Incubation with 1 $\times$ 10⁹ AmOMV particles increased CD11b⁺CD64⁺ cell frequency (2.31% to 17.18%) and IL-10 production compared to the medium group. Gene expression analysis revealed upregulation of *Adgre1* and *Nos2*, and downregulation of *Arg1* and *Mrc1* at the highest AmOMV concentration versus RA. These findings suggest that AmOMV promotes the differentiation of bone marrow-derived cells into tolerogenic M2 macrophages, underscoring their immunoregulatory potential in metabolic inflammation and T2D.

Financial support: CAPES, CNPq and FAPESP 24/06798-9.

Keywords: probiotic;M2 macrophage;anti-inflammatory cytokines.

3451 - Category: IMMUNOREGULATION (IR)

The impacts of a hyper caloric diet during severe polymicrobial sepsis

AMARAL, B.S.; SANTOS, B.M.D.A.; RIBEIRO, V.G.; OLIVEIRA, S.N.; SILVA, M.E.F.; AZEVEDO, L.M.S.; DINIZ, L.N.A.; BABETTO, A.M.; LUCAS, L.M.B.; FERREIRA, A.V.M.; TEIXEIRA, M.M.; DE SOUZA, D.D.G.. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Sepsis is an organ dysfunction caused by an unbalanced inflammatory host response to an infection that leads to tissue damage, septic shock and death. The microbiota is crucial in immune responses, but its role in sepsis remains unknown. Diets, such as a hypercaloric, can directly modulate the microbiota composition to a more inflammatory profile. Therefore, this study aimed to assess whether hypercaloric diets worsen sepsis. In order to evaluate this, C57BL/5 mice (8-12 weeks) were subjected to a hypercaloric (HC) and chow diet (DC) for 14 days, and after this time, sepsis was induced by CLP model. After surgery, animals were treated with Imipenem (10mg/kg) for 3 days. 24h before CLP, mice were submitted to a glucose tolerance test and were euthanized 6h and 3 days post-CLP with organ and fluid collection. Regarding the diet, HC mice presented higher glucose levels 15 minutes after glucose administration. Nevertheless, at 6h and 3 days post-CLP, HC mice showed a higher body fat percentage. Analyses on inflammatory parameters at 6h post CLP, showed leukopenia in blood and peritoneal cavity, and an increase of neutrophil activity in the lungs of CLP groups regardless of the diet. However, CLP HC showed more bacterial burden. On day 3, animals from both diets showed 60-65% of mortality, although CLP animals showed more bacterial burden and more neutrophil activity in the lungs. Altogether, these results suggest that the hypercaloric diet is capable of causing metabolic alterations in C57BL/5 mice, and support the hypothesis that changes in the microbiota, such as diets, are directly related to immune status after sepsis. As a perspective, mice will be subjected to a high-fiber diet post-CLP in order to evaluate if the diet changing is capable of reverting the inflammatory profile seen by the HC diet.

Keywords: Diet;Sepsis;Inflammation.

3454 - Category: IMMUNOREGULATION (IR)

Immunomodulatory effects of concentrated pitaya extract: in vitro, ex vivo, and preclinical colitis animal model

BOVI, W.G.; DESIDÉRIO, C.S.; SILVA, G.T.; MORAES, S.K.; LAMOGIA, A.S.A.; BRANDAO, S.G.; DE MAGALHÃES, P.C.; SILVA, A.L.C.A.E.; DE OLIVEIRA, C.J.F.. UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO, UBERABA - MG - BRASIL.

Chronic inflammatory diseases (CIDs), such as Inflammatory Bowel Disease (IBD), pose an increasing global public health challenge. IBD, which includes Crohn's Disease (CD) and Ulcerative Colitis (UC), is characterized by persistent and dysregulated inflammation of the gastrointestinal tract, with rising prevalence worldwide, including in Brazil. Its multifactorial etiology involves immune, genetic, and environmental factors, resulting in an imbalance between pro-inflammatory and regulatory mechanisms. Although current therapies exist, none are curative, highlighting the need for novel treatment strategies. In this context, the present study evaluated the therapeutic potential of pitaya pulp extract in colitis models. Cell viability assessed by resazurin assay in Vero cells confirmed the extract's safety (IC₅₀: 1/12.2). In RAW 264.7 macrophages, the extract directly modulated basal cytokine production, consistently reducing IL-6 and dose-dependently decreasing TNF- α , while increasing IL-1 β at certain dilutions. Ex vivo analysis using murine splenocyte explants revealed that the 1/30 dilution significantly increased IL-10 and decreased MCP-1 levels, indicating a complex, cell context-dependent immunomodulatory effect. The in vivo dextran sulfate sodium (DSS)-induced colitis model confirmed the extract's therapeutic potential, with the 1/10 dilution (DP10) optimizing reduction of clinical symptoms. Systemic and local cytokine analyses corroborated these benefits, showing decreased IL-6 and TNF- α and increased IL-10 in serum. In the intestine, reduced IL-1 β , IL-6, and IFN- γ suggested modulation of local inflammatory responses. These findings indicate that pitaya pulp extract is a promising source of bioactive molecules for new IBD therapies, acting through modulation of inflammatory responses and restoration of immune homeostasis.

Keywords: Colitis;Inflammation;Hylocereus.

3493 - Category: IMMUNOREGULATION (IR)

Cannabidiol and Δ^9 -Tetrahydrocannabinol as Immunomodulatory Agents: Insights from IL-1 β , IL-6 and TNF- α Modulation in peripheral blood mononuclear cellsDESIDÉRIO, C.S.¹; BOVI, W.G.²; SILVA, G.T.²; MORAES, S.K.²; SILVA, A.L.C.A.E.²; LAMOGIA, A.S.A.²; BRANDAO, S.G.²; DE OLIVEIRA, C.J.F.². 1. UFTM/UNIFACTHUS, UBERABA - MG - BRASIL; 2. UFTM, UBERABA - MG - BRASIL.

Cannabidiol (CBD) and Δ^9 -tetrahydrocannabinol (THC) are the main phytocannabinoids from *Cannabis sativa*, both reported to exert significant effects on immune function. While CBD acts through cannabinoid and non-cannabinoid targets, influencing immune cell activation, oxidative stress, and transcriptional regulation, THC primarily engages CB1 and CB2 receptors, modulating both innate and adaptive responses. Evidence indicates that these compounds can alter monocyte, macrophage, and T lymphocyte activity, impacting the release of inflammatory mediators. Such properties position CBD and THC as candidates for therapeutic modulation of immune responses. In this study, we evaluated the effects of a formulation containing CBD (300 mg) and THC (300 mg) on peripheral blood mononuclear cells (PBMCs) from healthy donors. Cells were exposed to concentrations of 0.1, 1, 10, and 100 $\mu\text{g/mL}$, and culture supernatants were collected after 24 h for cytokine quantification by enzyme-linked immunosorbent assay (ELISA). A concentration-dependent modulation of interleukin-1 (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor (TNF- α) was observed, with the 10 $\mu\text{g/mL}$ treatment showing the most pronounced positive modulation in cytokine production compared to untreated controls. These findings suggest that CBD and THC, in combination, can enhance the production of key inflammatory mediators in human immune cells, which may have implications in contexts where stimulation of immune responses is desirable, such as certain infectious diseases, cancer immunotherapy, and vaccine adjuvant development. Keywords: Cannabidiol;Cytokines; Δ^9 -tetrahydrocannabinol.

3495 - Category: IMMUNOREGULATION (IR)

Evaluation of the Antibacterial and Immunomodulatory Potential of a Curcumin-Loaded Nanostructured Polymer

LAMOGIA, A.S.A.; BRANDAO, S.G.; SILVA, A.L.C.A.E.; BOVI, W.G.; MORAES, S.K.; DE OLIVEIRA, C.J.F.; DESIDÉRIO, C.S.. UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO, UBERABA - MG - BRASIL.

Staphylococcus aureus is a common skin colonizer and a leading cause of cutaneous and soft tissue infections, and its growing antibiotic resistance has reduced treatment efficacy over recent decades. This issue has prompted the search for alternative therapies that combine antimicrobial, anti-inflammatory, and wound-healing effects. Curcumin, derived from *Curcuma longa* L., offers these properties but is limited by low solubility, stability, and bioavailability. In this context, nanotechnology, particularly polymeric nanowires, can enhance drug delivery through high surface area and controlled release. This study explored cellulose acetate and lignin-based polymeric nanowires enriched with curcumin as a potential strategy to combat *S. aureus* skin infections while modulating inflammation and promoting tissue repair. Electrospinning was performed using a solution containing cellulose acetate, dimethylacetamide, acetone, and lignin, forming nanofibers whose morphology was analyzed by scanning electron microscopy (SEM), showing homogeneous formation of the nanofibers with fiber diameters within the expected range. In vitro assays demonstrated the nanowires' safety in Vero cells, its immunomodulatory effects on RAW 264.7 macrophages, consistently increasing IL-10 production, and a mild, dose-dependent inhibitory effect on *S. aureus* growth. These findings indicate that the nanofibers show potential for biological applications, however, further investigations are needed to better understand its mechanisms and to optimize its formulation for future therapeutic applications.

Keywords: Curcumin;Nanotechnology;Staphylococcus aureus.

3497 - Category: IMMUNOREGULATION (IR)

Preparation of Polyvinyl Alcohol and Lignin Micro/Nanofibers Enriched with Neomycin Sulfate: Evaluation of Immunomodulatory and Antimicrobial Properties

BRANDAO, S.G.; LAMOGIA, A.S.A.; SILVA, A.L.C.A.E.; BOVI, W.G.; DESIDÉRIO, C.S.; MORAES, S.K.; OLIVEIRA, C.J.F.. UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO, UBERABA - MG - BRASIL.

The skin is a barrier against microorganisms, but skin infections remain a challenge, exacerbated by the inappropriate use of antibiotics and growing antimicrobial resistance. In this context, nanotechnology has emerged as a therapeutic alternative, especially with the development of micro/nanowires based on polyvinyl alcohol (PVA) and lignin—biocompatible and biodegradable polymers—incorporated with antimicrobials such as neomycin sulfate. In this context, the present study aimed to develop PVA and lignin (PVA + LIG) membranes, associated or not with neomycin sulfate, obtained by electrospinning, with the aim of evaluating their immunomodulatory, antimicrobial, and cytotoxic properties. Electrospinning was performed using a solution containing polyvinyl alcohol (PVA) and lignin, forming nanofibers and the morphology was analyzed by scanning electron microscopy (SEM), showing homogeneous formation of the nanofibers with fiber diameters within the expected range. Cell viability was measured in VERO epithelial cells using the resazurin assay (24 h). The immunological profile was evaluated in RAW 264.7 macrophages by quantifying IL-6, IL-10, and TNF via ELISA, and the antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* was determined by growth assay. The results demonstrated that PVA and PVA+LIG maintained cell viability >70%, while neomycin sulfate preserved >95% viability at concentrations of 0.23–15 mg/mL, with a significant reduction only at 30 mg/mL. An increase in TNF was observed, suggesting stimulation of a pro-inflammatory profile. In antimicrobial assays, neomycin-containing membranes promoted robust inhibition of bacterial growth. It is concluded that these membranes exhibit antimicrobial effects, pro-inflammatory effects, and low cytotoxicity, constituting promising alternatives for topical application in the management of skin infections and as an adjuvant strategy in combating bacterial resistance.

Keywords: Nanotechnology;Antimicrobial resistance;Polymers.

3519 - Category: IMMUNOREGULATION (IR)

EFFECTS OF OSTEOBLAST-DERIVED MICROVESICLES ON OSTEOCLAST DIFFERENTIATION AND FUNCTION

DA SILVA, A.C.R.. FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO (FCFRP-USP), RIBEIRÃO PRETO - SP - BRASIL.

Objectives: Investigate the effects of osteoblast-derived microvesicles (MVs) on the differentiation and function of osteoclasts *in vitro*. **Methods and Procedures:** Osteoclasts were obtained from bone marrow stem cells of C57BL/6 mice (CEUA Protocol N° 24.1.77.60.2) and differentiated in osteoclastogenic medium (Boyle, Simonet & Lacey, 2003). Microvesicles (MVs) were isolated from the MC3T3-E1 pre-osteoblast cell line on the 14th day of differentiation (Buchet et al., 2013). The influence of MVs on osteoclast differentiation was assessed by histochemical staining of TRAP-positive cells on day 5, using different MV concentrations. Bone resorption activity was evaluated in mature osteoclasts in the presence of 10 µg/mL MVs. **Results:** In the histochemical assay, the presence of MVs inhibited the formation of TRAP-positive cells and multinucleated giant cells (≥3 nuclei). In the bone resorption assay, osteoclasts exhibited a change in the resorption pattern in the presence of MVs. While osteoclasts normally resorb in a trench-like pattern, in the presence of MVs, bone resorption occurred in a pit-like pattern (Søe, Merrild & Delaissé, 2013). **Conclusions:** The data suggest that MVs not only interfere with the differentiation process but also modulate the resorptive profile of mature osteoclasts. **Acknowledgments:** This work was supported by the São Paulo Research Foundation (FAPESP) process n° 2023/12550-7; 2019/08568-2; 2013/08216-2.

Keywords: osteoclast;microvesicles ;osteoblast.

2667 - Category: INNATE IMMUNITY (IN)

Phenotypic Alterations in CD161⁺ T Cells are Associated with Severity in COVID-19 Patients

CARVALHO, N.O.; DE OLIVEIRA, G.; DOS SANTOS, T.M.A.F.; MIRANDA, V.H.S.; ALVES, C.C.; PEREIRA, A.A.S.; PRADO, R.O.; CARVALHO, K.A.B.; CAMPI-AZEVEDO, A.C.; PASCOAL-XAVIER, M.A.. INSTITUTO RENE RACHOU, BELO HORIZONTE - MG - BRASIL.

COVID-19 disease severity is associated with lymphopenia and distinct phenotypic and functional alterations. CD3⁺CD161⁺ T cells predominantly comprise unconventional T cell subsets and are crucial for immune responses due to their potent cytotoxic activity and rapid TCR-independent activation. Here, we evaluated the phenotypic profile of activation, cytotoxicity, and exhaustion of these cells by flow cytometry in blood samples of 86 RT-PCR-confirmed COVID-19 patients (at D0, D7, and D14-28 after study admission) and 12 unvaccinated healthy controls (HC). In patients, CD161⁺ T cell frequency was reduced in blood and presented a progressive increase in activated (CD38⁺, CD69⁺), cytotoxic (CD107a⁺, CD56⁺NKG2D⁺) and exhausted (PD-1⁺, LAG-3⁺) phenotypes, sustained up to D14-28. At D0, these phenotypes correlated with a higher frequency of naive memory cells (CD62L⁺, CD45RO⁺CD27⁺), while at D14-28, effector memory cells predominated. Stratification by CD4/CD8 subsets revealed that CD161⁺CD8⁺ T and CD161⁺CD4⁺CD8⁻ T subsets were most affected, with alterations in activation/cytotoxic and exhaustion phenotypes. Patients with fatal outcome exhibited distinct profiles *versus* discharged patients, with elevated frequencies of CD69⁺, CD107a⁺, and LAG-3⁺ cells. At D7, patients presented higher connectivity between immunological markers than HC group, indicating a critical point in disease progression. CD3⁺CD161⁺, CD161⁺CD56⁺NKG2D⁺ and CD161⁺PD-1⁺ cell subsets showed high accuracy (AUC ≥ 0.9) in distinguishing patients from HC, with CD161⁺PD-1⁺ and CD161⁺CD62L⁺ maintaining high discriminatory performance. However, the prognostic value of these phenotypes was moderate (AUC ≥ 0.68) in distinguishing discharge from death. These findings indicate that phenotypic alterations in CD161⁺ T cells are associated with COVID-19 severity and highlight their potential as immunological biomarkers for therapeutic strategies, T cell-based vaccine development, clinical monitoring of COVID-19 patients.

Keywords: COVID-19;; CD161+ T;unconventional T cells.

2707 - Category: INNATE IMMUNITY (IN)

ACOD1-Mediated Itaconate Pathway Enhances Host Resistance to Apicomplexan Parasites

DA SILVA, J.V.E.¹; LOPES, M.F.S.¹; TORRES, J.D.A.¹; MINEO, T.W.P.²; MOTA, C.M.¹. 1. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL; 2. UNIVERSIDADE FEDERAL UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL.

The Acod1 gene (aconitate decarboxylase 1) plays a key role in immunometabolism by converting cis-aconitate into itaconate in the citric acid cycle. Itaconate inhibits succinate dehydrogenase (SDH), leading to succinate accumulation and increased reactive oxygen species (ROS) production—mechanisms associated with anti-inflammatory effects and control of intracellular pathogens. This study investigated the role of Acod1 in host immune responses during infection by *Toxoplasma gondii* and *Neospora caninum*. Bioinformatic analysis of RNAseq data obtained from murine bone marrow derived macrophages (BMDM) demonstrated significant increase in the expression of the Acod1 gene in cells infected with either parasite. We further validated these findings with in vitro and in vivo experimental setups, confirming increased Acod1 expression upon infection with *T. gondii* or *N. caninum*. To further explore the role of ACOD1 in host resistance against these infections, we generated Acod1-deficient (Acod1^{-/-}) lines of murine fibroblasts and dendritic cells using CRISPR/Cas9 system. Both parasites presented increased replication rates in Acod1^{-/-} cells compared to WT controls. This reduction in the control of the parasite burden was associated with a reduction in ROS production in Acod1^{-/-} cells, a critical component of host restriction of parasite growth, explaining the heightened susceptibility of these genetically deficient cell lines to the infection. Altogether, our results highlight the importance of the Acod1-itaconate pathway in promoting ROS-mediated immune defense against *T. gondii* and *N. caninum*.

Keywords: Acod1;CRISPR-CAS9;Toxoplasma gondii.

2729 - Category: INNATE IMMUNITY (IN)

Increased IL-9 expression and Mast Cell induction by *L. lactis*-HSP65 in the colon: is related with Immunomodulatory Effects in Type 1 Diabetes

AVELINO, S.E.P.¹; OLIVEIRA, J.E.¹; NAKAMA, R.P.¹; ALVES, B.S.D.L.¹; PARDIM, M.F.¹; RIOS, W.M.¹; AZEVEDO, V.A.D.C.²; SARTORI, D.C.¹. 1. UNIVERSIDADE DE SÃO PAULO (USP), RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Type 1 diabetes (T1D) is an autoimmune disease marked by the immune-mediated destruction of pancreatic beta cells, leading to reduced insulin secretion and chronic hyperglycemia. Increasing evidence highlights the role of the gut in T1D pathogenesis, particularly the disruption of intestinal barrier integrity, decreased mucus production, and gut microbiota imbalance. These changes can exacerbate immune responses and favor the development of autoimmunity. Probiotic therapy has gained attention as a strategy to restore gut homeostasis and modulate immune function. In this study, we evaluated the effects of a recombinant strain of *Lactococcus lactis* genetically engineered to express heat shock protein 65 (HSP65), a molecule with anti-inflammatory properties in autoimmune conditions. Mice were administered 40 mg/kg streptozotocin (STZ) to induce diabetes and treated orally with *L. lactis*-HSP65 (LL-HSP65) at 1×10^9 CFU/day. Treatment began before and continued during disease induction, followed by administration every other day for 10 additional days. Mice receiving LL-HSP65 showed lower diabetes incidence and improved glycemic control compared to untreated diabetic controls. Both wild-type and LL-HSP65 strains significantly increased colonic mucus production. Although Muc1 and Muc2 gene expression did not differ significantly, an upward trend was observed in LL-HSP65-treated animals. Additionally, IL-22 expression was elevated in both probiotic groups, while IL-9 levels were significantly higher only in the LL-HSP65 group, indicating a potential protective role of this cytokine in mucosal immunity. We also observed increased colonic expression of mast cell markers Mmcp1 and Mmcp4, suggesting enhanced mast cell presence or activity. In conclusion, LL-HSP65 treatment leads to elevated IL-9 and mast cell markers, promoting mucus production and intestinal barrier reinforcement, which together contribute to improved immune regulation and protection against T1D.

Keywords: Diabetes type 1; Mast cells; Probiotic.

2803 - Category: INNATE IMMUNITY (IN)

Single-cell transcriptomic Profiling Reveals the Impact of Aging on Human Skin Cell Populations

YABUUTI, E.M.¹; RODOVALHO, V.D.R.²; RODRIGUES, H.G.¹. 1. UNIVERSITY OF CAMPINAS, LIMEIRA - SP - BRASIL; 2. NATIONAL LABORATORY OF SCIENTIFIC COMPUTING, PETRÓPOLIS - RJ - BRASIL.

Skin types exhibit particularities in their response to treatments. Advances in omics sciences have contributed to a better understanding of tissue heterogeneity, including transcriptomic and cellular composition variations. However, the molecular mechanisms underlying immune responses particularly under perturbations, remain to be further explored. This study aimed to characterize the transcriptomic profile of human skin during aging and to establish adaptable computational pipelines for future analyses. For this, we used a public Single-cell RNA sequencing (scRNA-seq) dataset (HRA000395) obtained from the National Genomics Data Center - GSA consisting of eyelid skin samples from nine healthy female donors classified into three age groups: Young, Middle-aged, and Old. Based on previous findings indicating that transcriptomic differences are most evident between the Young and Middle-aged groups, only these were included in the present analysis, following the methodology of Heumos et al. (2023). Briefly, quality control involved filtering outliers using median absolute deviations (MAD) thresholds and excluding cells with high mitochondrial or hemoglobin expression. Data were normalized with Scran, and clustering was performed using the Leiden algorithm, allowing the annotation of eleven cell types based on literature-derived marker genes. Differential expression analysis was conducted using the MAST method. Notably, the transcription factors *HES1* in fibroblasts and *KLF6* in keratinocytes, both with geroprotective roles, were found to be downregulated in Middle-aged individuals compared to the Young group. This study enabled the identification of 10 out of the 11 cell types highlighted in the original publication, in addition to a cluster of lymphatic endothelial cells that had not been annotated in that study.

Keywords: Single-Cell RNA transcriptomics; Human skin; Bioinformatics tools.

2811 - Category: INNATE IMMUNITY (IN)

Profile of cutaneous innate lymphoid cells following exposure to *Aedes aegypti* mosquito bites in naive and sensitized hosts

DA SILVA, A.F.; DA FONSECA, D.M.; NUNES, A.D.S.. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Aedes aegypti, is the mosquito vector of arboviruses such as dengue, Zika, chikungunya, and yellow fever. During the blood feeding, the mosquito injects saliva into the host which contains bioactive molecules with anti-hemostatic and immunomodulatory properties that can modulate various immune cells. In a previous work, our group characterized the cutaneous inflammatory infiltrate following mosquito bites and observed that mast cells, eosinophils, and CD4⁺ T lymphocytes are the predominant cells in sensitized animals. In addition elevated production of IL-4, IL-5, and IL-13 in the bronchoalveolar lavage and lungs, as well as mucus secretion, were observed in a model of sensitization and pulmonary challenge, indicating the involvement of a type 2 immune response. Based on these findings, we hypothesized that innate lymphoid cells (ILCs), particularly ILC2, may play a role in the immune response to *Ae. aegypti* salivary components. ILCs lack antigen receptors but function similarly to helper T cells. Therefore, our aim was to characterize ILCs in saliva-sensitized (SGE) and non-sensitized (PBS) mice exposed to mosquito bites. Using multiparametric spectral flow cytometry, we observed an increase in the absolute number of CD45⁺ cells in the SGE group at 6 and 24 hours and in the PBS group at 6 hours, suggesting enhanced immune cell migration and/or proliferation at the mosquito bite site. We also found increased expression of the transcription factor GATA3 at 24 hours after the bites, within both CD4⁺ T cells and ILCs, suggesting the activation of Th2 and ILC2 populations, respectively, in the SGE and PBS groups. Additionally, FoxP3 expression in CD4⁺ T cells was significantly elevated in the PBS group at 24 hours and in the SGE group at 6 and 24 hours when compared to baseline levels (steady-state). To date, no studies have correlated these populations with *Ae. aegypti* salivary components.

Keywords: *Aedes aegypti*; Innate lymphoid cells; Skin.

2826 - Category: INNATE IMMUNITY (IN)

Latent Cytomegalovirus Infection Selectively Enhances IL-1 β Response to Acute Exercise in Physically Active Men

PERES, A.¹; DA SILVA, I.M.²; ROMÃO, P.R.T.²; DORNELES, G.P.³; SANTOS, M.A.L.²; SCHIPPER, L.D.L.²; BOEIRA, M.D.R.¹; LIRA, F.S.⁴. 1. UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE, PASSO FUNDO - RS - BRASIL; 2. UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL; 3. SOCIAL RESPONSIBILITY. HOSPITAL MOINHOS DE VENTO, PORTO ALEGRE - RS - BRASIL; 4. UNIVERSIDADE DO ESTADO DE SÃO PAULO, PRESIDENTE PRUDENTE - SP - BRASIL.

Exhaustive physical exercise may transiently increase susceptibility to infections, especially of the upper respiratory tract. This phenomenon is influenced by factors such as sleep, nutritional status, and training load. Recently, latent viral infections have been proposed as modulators of the immune response to exercise, but this association remains poorly understood. This study aimed to investigate whether latent cytomegalovirus (CMV) infection influences inflammatory and purinergic responses to a single session of high-intensity interval exercise (HIIE). We recruited 22 healthy, physically active men (25.3 ± 1.4 years; VO_{2max} : 48.25 ± 6.18 mL/kg/min), stratified into CMV-positive and CMV-negative groups based on IgG serostatus. Participants completed health and physical activity questionnaires, anthropometric measurements, and cardiopulmonary fitness assessments, followed by an HIIE protocol (10×1 min at 85–90% HR_{max}, interspersed with 75 s of active recovery at 50% HR_{max}). Blood samples were collected before and after exercise for analysis of plasma and PBMC responses. A significant interaction between time and CMV status was observed for lipopolysaccharide (LPS) and interleukin-1 β (IL-1 β): LPS increased in both groups, but IL-1 β increased only in CMV-positive individuals. No significant interactions were found for adenosine, ATP, ADP, AMP, prostaglandin E₂ (PGE₂), or creatine kinase (CK), although CMV-positive participants showed higher baseline PGE₂. CK increased post-exercise in both groups. These findings suggest that latent CMV infection enhances IL-1 β release following high-intensity exercise, highlighting a potential role of viral latency in modulating acute inflammatory responses in physically active individuals.

Keywords: Cytomegalovirus; Exercise immunology; Inflammation.

2843 - Category: INNATE IMMUNITY (IN)

Short-chain fatty acid acetate modulates neutrophil functional maturation and protects mice against DSS-induced colitis

DE OLIVEIRA, S.¹; FACHI, J.L.²; RODRIGUES, P.F.²; BHATTARAI, B.²; LAYDEN, B.T.³; GILFILLAN, S.²; COLONNA, M.²; VINOLO, M.A.R.¹. 1. UNIVERSITY OF CAMPINAS, CAMPINAS - SP - BRASIL; 2. WASHINGTON UNIVERSITY IN ST. LOUIS, SAINT LOUIS - ESTADOS UNIDOS DA AMERICA; 3. UNIVERSITY OF ILLINOIS CHICAGO, CHICAGO - ESTADOS UNIDOS DA AMERICA.

The intestinal microbiota is crucial for the generation of immune cells, modulating this process either directly through interaction with the host or indirectly via metabolites such as short-chain fatty acids (SCFAs). Studies have shown that acetate-FFAR2 signaling in neutrophils is essential for mucosal immunity. However, the role of this axis in the production and maturation of neutrophils remains uncertain. For investigating this, we used 8-week-old mice with conditional gene deletion and knockouts. Mice were either treated or not with acetate for 12 days and, during the last 6 days, they received 2.5% dextran sodium sulfate (DSS) in their drinking water for colitis induction. Acetate had a protective effect on colitis development, reducing body weight loss, as well as the clinical and histological alterations associated with the disease. Among the populations analyzed, we observe an increase in the number of neutrophils in bone marrow, peripheral blood, and colon lamina propria during inflammation. Acetate treatment also led to an expansion of myeloid progenitors in the bone marrow, and neutrophil depletion during inflammation worsened colitis severity. Acetate affected neutrophil production in the bone marrow in a manner partially dependent on FFAR2 signaling, with effects restricted to the final stages of differentiation, rather than the early phases of hematopoiesis. Additionally, we found that the protective effect of the metabolite depends on FFAR2 signaling and is lost in its absence. Collectively, our data supports a model in which the microbiota-SCFA-neutrophil axis influences host inflammatory responses by modulating neutrophil maturation.

Keywords: Short-chain fatty acids; Neutrophil; Inflammation.

2845 - Category: INNATE IMMUNITY (IN)

Functional and Metabolic Alterations in Plasmacytoid Dendritic Cells (pDCs) in Sickle Cell Anemia

PAES, I.F.; DE SOUZA, M.A.; COSTA, F.F.; ZUNIGA, E.; COSTA, R.S.. UNICAMP, CAMPINAS - SP - BRASIL.

Sickle cell anemia (SCA) is a hereditary hemoglobinopathy characterized by the polymerization of hemoglobin S, leading to erythrocyte deformation, intravascular hemolysis, and chronic inflammation. Patients and mice models of SCA exhibit gene signature of type I interferon (IFN-I) signaling. In this study, we investigated whether plasmacytoid dendritic cells (pDCs), the main producers of IFN-I, contribute to this signature by using Townes HbSS transgenic mice as an SCA model and HbAA wild-type mice as controls. The frequency and phenotype of pDCs was assessed by flow cytometry, and *in vivo* stimulation of pDCs was performed with CpGA iv. injection. Sorted pDCs were incubated with serum from HbAA or HbSS mice and CpGA for evaluation of IFN-I production by RT-PCR and ELISA, and cellular metabolism by flow cytometry using SCENITH protocol. Our data show that the number of pDCs was lower in bone marrow but higher in spleen and lymphnodes of HbSS mice when compared with controls. In addition, pDCs from all tissues showed an activated phenotype, as observed by higher Sca-1 expression. *In vivo* IFN- α production following CpGA stimulation was impaired in SS mice, suggesting functional exhaustion of pDCs. Accordingly, HbSS pDCs showed higher IFN- α expression in steady state, and, when pDCs were incubated with HbSS serum, they were not able to respond to a second stimulus with CpG-A, indicating refractoriness. Metabolically, SS pDCs from bone marrow showed higher mitochondrial dependence and reduced glycolytic capacity. pDCs treated with HbSS serum exhibited a similar phenotype, with decreased viability, suggesting that soluble factors in sickle serum induce this dysfunctional profile. Altogether, these data indicate that pDCs are, at least in part, responsible for IFN- α production in SCA, and that pDC stimulation is induced by factors present in the serum. Stimulation of pDC induces metabolic dysfunction, which may be, at least in part, related to exhausted phenotype of pDCs.

Keywords: Sickle cell anemia; plasmacytoid dendritic cells; IFN-I.

2848 - Category: INNATE IMMUNITY (IN)

Profile of dendritic cell and monocyte subsets and their correlation with clinical parameters in sickle cell anemia

DE SOUZA, M.A.¹; PAES, I.F.¹; COSTA, F.F.²; COSTA, R.S.². 1. INSTITUTE OF BIOLOGY – UNICAMP, CAMPINAS - SP - BRASIL; 2. HEMOCENTER – UNICAMP, CAMPINAS - SP - BRASIL.

Sickle cell anemia (SCA) is caused by a mutation in the gene that encodes beta globin, resulting in abnormal hemoglobin that leads to chronic hemolysis, inflammation, and immune dysregulation. Using flow cytometry on PBMCs from SCA patients and healthy individuals, we analyzed dendritic cell (DC) and monocyte (Mo) subpopulations. Unsupervised clustering analyses showed that cDC1 were reduced in patients and showed elevated CD40 and CD71 expression, suggesting lower circulation and higher activation. Within the pDCs group, one cluster, marked by high Sirp- α , was significantly increased in SCA patients. cDC2 subsets were heterogeneous, with one cluster, marked by low Sirp- α and high CD40, increased in patients, indicating an activated phenotype. All DC subsets, except tDCs had higher HO-1 expression in SCA patients. Clustering of Mo populations revealed a reduced cluster of classical Mo in SCA patients, which express high Sirp- α . Intermediate and non-classical Mo had stable frequencies but higher HO-1 expression. Correlational analyses showed total DCs were positively associated with leukocyte and neutrophil counts, CD4⁺ T cell activation, and Treg frequency. cDC1, pDC, and iDC correlated with increased IFN- γ and IL-17 from T cells, while cDC2 showed inverse association. HO-1 expression in DCs and Mo correlated positively with inflammatory cytokines, reticulocytes, and hemolysis parameters, and negatively with hemoglobin and hematocrit, showing the role in sensing anemia. Sirp- α expression was reduced in hemolytic conditions, suggesting loss of inhibitory signaling. CD71 expression in DCs and Mo correlated with bilirubin and Th17 activation, suggesting a link between iron uptake and inflammation. Altogether, our data expand the knowledge about the activation of DCs and their roles on T cell dysfunction presented by SCA patients. Additionally, the data point out the correlation of heme and iron metabolism by DCs and Mos with clinical diseases parameters.

Keywords: Dendritic cell; Monocyte; Sickle cell anemia.

2856 - Category: INNATE IMMUNITY (IN)

Neutrophil Extracellular Traps (NETs) induce monocyte activation through NF- κ B and TLR2 signaling pathways

ROCHAEL, N.C.¹; GONÇALVES, B.S.¹; DA SILVA, M.D.S.T.¹; BOU-HABIB, D.C.¹; SARAIVA, E.². 1. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Neutrophils and monocytes are essential cells for the generation of inflammatory reactions, interacting with each other in inflamed sites. Neutrophils modulate the function of other cells via their mediators, including NETs, which are molecular structures formed by DNA decorated with proteins. The functional impact of the interaction between NETs and monocytes has not been studied up to now. Thus, here we assessed if NETs activate monocytes, and analyzed the molecular pathways involved in the functional modulation of these cells. Human monocytes were treated with purified NETs released by neutrophils exposed to inactivated Sars-CoV-2 (inCoV2). After 24 hours, cell culture supernatants were analyzed for production of reactive oxygen species (ROS) and inflammatory mediators. NET-treated monocytes released significant amounts of ROS, the pro-inflammatory cytokines IL-6 and TNF- α , and the β -chemokines MIP-1 α and RANTES. We next analyzed whether the transcription factor NF- κ B was involved in this process, by treating monocytes with the NF- κ B inhibitor BAY 11-7082 thirty minutes before cell exposure to NETs. We found that blocking NF- κ B signaling reduced the production of TNF- α , IL-6 and MIP-1 α , suggesting that NETs activate monocytes through this molecular pathway. Next, we evaluated the role of TLR2 receptor in NET-mediated monocyte activation by treating these cells with the TLR2 inhibitor C29 thirty minutes before exposure to NETs. We found that blockade of TLR2 receptor reduced the production of IL-6, suggesting the engagement of this receptor in NET-mediated monocyte activation. Finally, NET-treated monocytes showed increased levels of activation markers CD38, CD86, and HLA-DR by flow cytometry. Taken together, our results show that NETs promote monocyte activation, inducing a pro-inflammatory profile. Further studies are in progress to search for other molecular mechanisms participating in the NET-mediated monocyte activation, including the engagement of TLR4.

Keywords: Neutrophil extracellular traps; monocyte activation; NF κ B and TLR2 signaling.

2867 - Category: INNATE IMMUNITY (IN)

Experimental Validation of hsa-miR-503-5p Target Genes in a THP-1 Derived Macrophage Cell Model

FRACAZZO, M.C.C.¹; CASTRO, R.²; DE LIMA, J.O.²; DAMAS, M.S.F.²; SUGIYAMA, F.H.C.²; FONTANARI, C.²; FRANTZ, F.G.³. 1. SCHOOL OF MEDICINE OF RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL; 2. SCHOOL OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL; 3. FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO (USP), RIBEIRÃO PRETO - SP - BRASIL.

MicroRNAs (miR) are small endogenous RNA that influence various important biological processes, including gene suppression, mRNA splicing, and the activation or inactivation of transcription factors, among others. There is experimental and predicted evidence that miR-503-5p presents a role as a regulator of several pathways related to cell cycle progression, but our hypothesis is that miR-503-5p is involved in senescence pathways during HIV infection. We conducted a bioinformatic analysis to investigate how miR-503-5p could contribute to cellular senescence. Combining several prediction tools, such as TarBase v9.0, miRTargetLink 2.0, miRDB, and miRWalk, and setting a confidence threshold of 7.0 we gathered an initial pool of 7,896 genes potentially regulated by miR-503-5p and used R® software to pinpoint the common genes across all platforms. By comparing these genes with known cell cycle and senescence pathways from KEGG Pathways, we identified promising targets for experimental validation. The experimental approach involved differentiating THP-1 cells into macrophage-like cells using 100 ng/mL of PMA for 72 hours. Following differentiation, cells were transfected with miR-503-5p mimic for 48 hours using Lipofectamine™ iMAX®. The overexpression of miR-503-5p was confirmed by TaqMan qPCR, and the expression levels of targeted genes were evaluated by RT-qPCR. The results indicate that the method of transfection was effective ($p < 0.05$) and we were able to observe the effects of miR-503-5p in modulating important genes related to the senescence pathway. In conclusion, our results suggest that miR-503-5p may play a regulatory role in the expression of key cell cycle checkpoint genes in THP-1 derived macrophages and could be an important factor in the induction of dysfunctions related to impaired cell cycle progression. Future studies will explore cytokine production and gene expression contributing to cellular senescence and cell cycle dysfunction.

Keywords: immunosenescence; bioinformatics; epigenetics.

2872 - Category: INNATE IMMUNITY (IN)

Neutrophil Extracellular Traps promote macrophage activation through Toll-like receptor engagement and PKA and NF-κB signaling pathways

GONÇALVES, B.S.¹; QUESNE, A.H.M.L.²; TEMEROZO, J.¹; ROCHAEL, N.C.³; DE AZEVEDO, S.S.D.⁴; BELLO, G.⁴; ALVES, M.R.⁵; SARAIVA, E.⁶; BOU-HABIB, D.C.¹. 1. LABORATORY ON THYMUS RESEARCH, OSWALDO CRUZ INSTITUTE/FIOCRUZ AND POSTGRADUATE PROGRAM IN CELLULAR AND MOLECULAR BIOLOGY, OSWALDO CRUZ INSTITUTE/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. LABORATORY ON THYMUS RESEARCH, OSWALDO CRUZ INSTITUTE/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. LABORATORY ON THYMUS RESEARCH, OSWALDO CRUZ INSTITUTE/FIOCRUZ AND LABORATORY OF IMMUNOBIOLOGY OF LEISHMANIASIS, FEDERAL UNIVERSITY OF RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 4. LABORATORY OF AIDS AND MOLECULAR IMMUNOLOGY, OSWALDO CRUZ INSTITUTE/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 5. EVANDRO CHAGAS NATIONAL INSTITUTE OF INFECTOLOGY, HIV/AIDS CLINICAL RESEARCH CENTER/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 6. LABORATORY OF IMMUNOBIOLOGY OF LEISHMANIASIS, FEDERAL UNIVERSITY OF RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL.

Neutrophils have key roles in innate immune responses and can release extracellular traps (NETs), characterized by chromatin exteriorization associated with cytoplasmic and granule proteins, such as neutrophil elastase, myeloperoxidase, HMGB1 and S100 family. These traps are released upon neutrophils' contact with several factors, including inflammatory mediators and infectious agents. Since NET-macrophage interaction occurs in different tissues and in a physiological context, we aim to elucidate the impact of this interaction on cellular activation. Human monocyte-derived macrophages from healthy donors were exposed to NETs induced by inactivated SARS-CoV-2 (inSARS) or inactivated HIV-1 (inHIV) and the production of inflammatory mediators was analyzed. NETs increased macrophage production of reactive oxygen species and promoted NF-κB activation. Furthermore, NETs induced the release of the β-chemokines MIP-1α, MIP-1β and RANTES, as well as IL-6, IL-8, IL-10 and TNF-α. To investigate receptors engaged in NET recognition and cellular activation, we used pharmacological inhibitors of Toll-like receptor (TLR) 2 and 4. The inhibition of TLR2, TLR4 and NF-κB signaling abrogated macrophage production of inflammatory mediators induced by NETs. NET-mediated macrophage activation was also reduced upon inhibition of protein kinase A and blockade of actin polymerization with Cytochalasin D, suggesting that both pathways are required for NETs effect. RNA sequencing revealed that thirty-three genes were differentially expressed, with sixteen biological processes and two molecular functions enriched in NET-treated macrophages. Our findings show that NETs modulate macrophage transcriptional profile and functioning through TLR2 and TLR4 engagement and PKA and NF-κB recruitment.

Keywords: Macrophages; NETs; Cellular Activation.

2877 - Category: INNATE IMMUNITY (IN)

Impact of metabolism in the modulation of CD1d expression in hepatoma cells

LABATE, J.D.L.; DE QUEIROZ, A.M.; KELLER, A.C.. UNIFESP, SÃO PAULO - SP - BRASIL.

Several pieces of evidence support a relationship between the levels of CD1d expression in the tumor and iNKT-mediated immunovigilance. Although glycolysis inhibition increases CD1d presentation by antigen-presenting cells, its effects on tumor cells remain unclear. Because our laboratory is interested in the modulation of CD1d by butyl-coumarate (b-pCA), this study aimed to determine the impact of glycolysis on the surface expression of CD1d in hepatoma cells, as well as on the ability of b-pCA to modulate this molecule. The murine hepatoma cell line Hepa-1c1c7 cultured in glucose-deprived medium exhibited a significant decrease in cell surface expression of CD1d. In contrast, treatment with b-pCA increased CD1d expression on the surface of Hepa-1c1c7 cells, both under normal conditions and under glucose deprivation. In conclusion, our data indicate that glycolysis plays an important role in maintaining the basal levels of CD1d expression in hepatoma cells, and that the b-pCA is a viable alternative for increasing CD1d expression in tumors under a glucose-deprived environment.

Keywords: Metabolism;CD1d;Hepatoma

2894 - Category: INNATE IMMUNITY (IN)

ASC-dependent inflammasome activation in allergic asthma induced in mice genetically selected for maximum and minimum acute inflammatory responseZANA, N.C.T.¹; DE OLIVEIRA, M.A.¹; FAVORETTO, B.C.²; TREZENA, A.G.²; DE FRANCO, M.T.²; IRINEU, M.D.O.²; RIBEIRO FILHO, O.G.²; DE LIMA, W.T.¹; STAROBINAS, N.². 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

Asthma is a chronic inflammatory disease of the airways. The inflammasome pathway is associated with allergic responses and consists of a multimeric protein complex formed by pattern recognition receptors, which recruit the ASC adaptor and activate caspase, leading to the release of IL-1 β and IL-18, and pyroptotic cell death. To explore the relationship between inflammasome activation and allergic asthma, we used mouse lines selectively bred for maximal (AIRmax) and minimal (AIRmin) acute inflammatory responses. These strains carry different alleles of the PYCARD gene, which encodes the ASC protein, representing phenotypic extremes of inflammation. Three lineages were used: AIRmax C/C, AIRmin C/C, and AIRmin T/T. The AIRmin T/T genotype transcribes an amino acid modification resulting in a nonfunctional ASC protein. Mice were sensitized with LPS-free ovalbumin (OVA) and alum on days 0 and 7, followed by intranasal OVA challenges on days 14 and 21. Pulmonary mechanics were analyzed 24 hours after the last challenge using the FlexiVent system. AIRmax C/C asthmatic mice showed significant increases in airway and tissue resistance, while AIRmin C/C showed increased tissue elastance compared to control. No changes were observed in AIRmin T/T asthmatic mice. Bronchoalveolar lavage (BAL) and lung tissue were analyzed for total and differential cell counts. AIRmax C/C and AIRmin C/C asthmatic mice presented decreased macrophages and increased eosinophils, while AIRmin T/T showed no changes. Immunophenotyping cells on day 22 confirmed these findings. Serum levels of anti-OVA IgG and IgG1 measured by ELISA were elevated in all asthmatic groups. In conclusion, asthma allergic induced in AIRmin mice bearing PYCARD T/T allele don't show alteration in lung mechanics and no difference of cell population in the lungs. Indicating a correlation between PYCARD genotype and allergic asthma.

Keywords: Asthma;Inflammasome;Acute Inflammation.

2898 - Category: INNATE IMMUNITY (IN)

Characterization of mast cell DNA extracellular traps (DETs) induced by SARS-CoV-2 and its effect in the Calu-3 virus infection

BARBOSA, B.C.D.N.¹; DA SILVA, G.C.P.¹; LIMA-GOMES, P.D.S.¹; ROCHAEL, N.C.¹; TEMEROZO, J.²; COELHO, S.V.A.¹; LOPES, P.O.³; DE ARRUDA, L.B.¹; COVRE, L.P.³; GOMES, D.C.D.O.³; BOU-HABIB, D.C.²; SARAIVA, E.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITORIA - ES - BRASIL.

Mast cells are immune sentinels strategically located in tissues that interface with the external environment, such as the skin and the mucosa of the respiratory and gastrointestinal tracts. These cells can exert both protective and detrimental roles during inflammation. In addition to their known effector functions, mast cells also display microbicidal activities, including phagocytosis and the release of extracellular traps composed of DNA associated to proteins (DETs). Neutrophil extracellular traps (NETs) have been detected in the plasma and tracheal aspirates of COVID-19 patients, where they contribute to disease pathology through cytotoxic effects on lung epithelial cells and thrombus formation. Similarly, we demonstrate here, that SARS-CoV-2 induces DET extrusion by the human mast cell line HMC-1, composed of a DNA scaffold decorated with H3 citrullinated histone, tryptase, LL-37, and β -hexosaminidase. TLR2 and TLR4 inhibition decreased DET extrusion by SARS-CoV-2. Signaling pathways involved in DET release include the generation of reactive oxygen species (ROS), intracellular calcium mobilization, activation of peptidylarginine deiminase (PAD), and tryptase. Purified DETs were cytotoxic toward pulmonary epithelial and endothelial cells in vitro, with histones and tryptase playing key roles in this effect. Moreover, we found that DETs decreased SARS-CoV-2 infection in alveolar epithelial Calu-3 cells, dependent of H3citrullinated histone, DNA scaffold integrity and tryptase. Mast cell DETs were evidenced in lung biopsies from COVID-19 patients, confirming their presence in vivo. Together, our findings highlight a novel role for mast cells in COVID-19 pathogenesis. DETs not only exert cytotoxic effects on lung epithelial and endothelial cells but also modulate viral infection and are present in the lungs of COVID-19 patients. These results suggest that DETs released by mast cells contribute to the lung damage observed in COVID-19 patients.

Keywords: Mast cells;SARS-CoV-2;COVID-19.

2905 - Category: INNATE IMMUNITY (IN)

Extracellular traps by mast cells: induction by *Leishmania amazonensis*

DA CONCEIÇÃO JUNIOR, F.; BARBOSA, B.C.D.N.; DA SILVA, G.C.P.; SARAIVA, E.. FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*, transmitted by female sandflies, which inject promastigotes into the skin where the promastigotes interact with different cells of the innate immune system, such as resident mast cells. These sentinel cells serve as an interface between the host and the external environment. Upon contact with *Leishmania*, mast cells can degranulate, release various inflammatory mediators and the DNA extracellular traps (DETs). DETs are composed of chromatin associated with various proteins capable of trapping and/or killing microorganisms. Here, we aimed to investigate whether the human mast cell line (HMC-1) release DETs when stimulated by *Leishmania amazonensis* promastigotes, evaluating the signaling pathways involved in this process using pharmacological inhibitors, and to assess DETs' toxicity to the promastigotes. Our results reveal that *L. amazonensis* promastigotes induce DETs in the HMC-1 mast cells, composed of a DNA scaffold associated with H3 citrullinated histone and tryptase. The release of these DETs is a mechanism dependent on reactive oxygen species, peptidyl arginine deiminase (PAD), calcium, and serine proteases. Although entrapped promastigotes were not killed by DETs. Our findings demonstrate that *Leishmania amazonensis* promastigotes trigger DET release in mast cells, expanding the understanding of early innate immune responses to leishmaniasis.

Keywords: Mast cell;Leishmaniasis;DNA extracellular Traps.

2907 - Category: INNATE IMMUNITY (IN)

Nicotinamide Adenine Dinucleotide (NAD) metabolism modulates the inflammatory response of Peripheral Blood Mononuclear Cells (PBMCs) stimulated with inactivated SARS-CoV-2

DA SILVA, M.D.S.T.; FORMIGA JUNIOR, M.A.; TEMEROZO, J.; BOU-HABIB, D.C.. INSTITUTO OSWALDO CRUZ (IOC/FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

Nicotinamide adenine dinucleotide (NAD) is an essential coenzyme for cellular redox reactions and a substrate for enzymes involved in immunometabolic regulation, known as NADases such as CD38, PARPs, and sirtuins. Evidence suggests that cellular metabolism, especially NAD metabolism, plays a crucial role in regulating innate immune responses. In this study, we investigate whether NAD metabolism regulates the activation and inflammatory profile of peripheral blood mononuclear cells (PBMCs) exposed to inactivated SARS-CoV-2 (inCoV-2), which has been shown to induce a robust inflammatory response. PBMCs and purified monocytes from healthy donors, obtained by density gradient and plastic adherence, respectively, were challenged with inCoV-2 at 0.01 or 0.1 PFU/mL during 12 or 24 hours. Flow cytometry was performed to evaluate CD38 expression, one of the main NAD-consuming enzymes, in CD3+ and CD14+ cells. NAD consumption and reactive Oxygen species (ROS) production were evaluated by enzymatic assays, and cytokine levels were measured by ELISA methods. Our findings show that inCoV-2 increased CD38 expression in monocytes and NAD+ degradation in PBMCs and monocytes, which was reverted by a selective CD38-NADase activity inhibitor. InCoV-2 also increased ROS generation and production of the cytokines IL-6, TNF, IL-1b, IL-8 and IL-10 in PBMCs. The inhibition of CD38 NADase activity abrogated the inCoV-2-mediated enhancement of IL-6 and IL-10, but did not control the augmentation of TNF, IL-1b, and IL-8. Our data suggest that inCoV-2 modifies NAD metabolism and that CD38 activity regulates, at least partially, the production of inflammatory mediators induced by inCoV-2. In our next steps, we will analyze the role of other NAD-consuming enzymes, as well as the precursors of this molecule, on the dynamics of NAD+ metabolism in the context of the SARS-CoV-2 inflammatory response in immune cells.

Keywords: NAD metabolism; Inflammation; SARS-CoV-2.

2916 - Category: INNATE IMMUNITY (IN)

Analysis of the trimethylation status of lysine 4 of histone H3 (H3K4me3) in the promoters of genes involved in trained immunity

DA SILVA, N.J.; MOSIMANN, A.L.P.; NARDELLI, S.C.. INSTITUTO CARLOS CHAGAS - FIOCRUZ PARANÁ, CURITIBA - PR - BRASIL.

In eukaryotes, gene expression regulation is refined and involves many different processes, from chromatin organization in the nucleus to the stability of the resulting protein in the cytoplasm. Chromatin represents the first barrier to all DNA-dependent processes. Both histones and DNA are targets of post-translational modifications (PTMs) that interfere with gene transcription. Currently, many modifications have been identified in different organisms, revealing their own histone codes, whose function is still unknown and which affect the regulation of gene expression. These mechanisms are encompassed in epigenetic regulation, that is, the alteration of gene expression that acts through the activation or repression of certain genes, but does not affect the DNA sequence. Cells involved in the immune response, like other cells in the body, are subject to transcriptional regulation mechanisms such as epigenetic ones. More recently, the importance of these mechanisms in the phenomenon of trained immunity has been demonstrated. Trained immunity is characterized by a faster and more efficient response of innate immune system cells to a second stimulus not necessarily related to the first. In the case of the trained immunity phenomenon, its direct link to epigenetic and metabolomic reprogramming induced in innate immune system cells after exposure to an antigen, such as BCG, for example, has already been demonstrated. In this context, we aim to assess the enrichment of the activating histone mark H3K4me3 in promoters of genes associated with trained immunity. So far, we have standardized the native chromatin immunoprecipitation (N-ChIP) protocol in RAW 264.7 cells, and primers to be used in qPCR have been validated by conventional PCR. The next steps involve applying the N-ChIP protocol to murine bone marrow-derived macrophages (BMDMs), allowing the analysis of H3K4me3 dynamics in a more physiologically relevant model.

Keywords: Epigenetic; Trained immunity; H3K4me3.

2926 - Category: INNATE IMMUNITY (IN)

Investigating the role of intestinal microbiota in innate memory induction

IGNACIO, A.¹; FERREIRA, M.D.²; CZYZ, S.³; KOVTONYUK, L.²; WINTHEREIK, L.³; MONTEIRO, L.D.B.⁴; GELTINK, R.K.⁵; MCCOY, K.³. 1. UNIVERSITY OF CALGARY, CALGARY - CANADA; 2. UNIVERSITY OF CALGARY, CALGARY - CANADA; 3. UNIVERSITY OF CALGARY, CALGARY - CANADA; 4. UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER - CANADA; 5. UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER - CANADA.

The profound importance of microbiota-host interactions in regulating immunity has been demonstrated in multiple illnesses. However, whether the gut microbiota can induce innate memory (also referred to as 'trained immunity') in innate cells remains elusive. Our group demonstrated that transient colonization of pregnant dams during pregnancy only results in germ-free (GF) offspring with an altered immune system landscape later in life, providing strong evidence of memory-like phenotype induced by intestinal microbiota. Therefore, ***I hypothesized that microbiota-derived products and metabolites act as a primary challenge, inducing long-lasting reprogramming of innate immune cells, resulting in protection from infections.***

METHODOLOGY: GF pups were gavaged with the auxotrophic *Escherichia coli*. Because this bacterium cannot survive more than 48h in vivo, the pups return to GF status, which allows the induction of innate memory to be disassociated from continuous microbial exposure. Control pups were gavaged with PBS. At 8 weeks of age, GF mice were infected with *S. aureus* or challenged with lipopolysaccharide. Immunophenotyping and bacterial burden were determined. Analysis of the bone marrow compartment was performed by colony-forming units assay and by bone marrow-derived macrophages (BMDM) assays.

RESULTS: Transient colonization in early life protects from *S. aureus* dissemination and increases the survival rate in adulthood. This protection is associated with increased monocyte counts. Transient colonization increased the hematopoietic stem cell (HSC) population, with higher longevity ex vivo. BMDM generated from transiently colonized mice showed decreased mitochondrial potential, increased mitochondrial ROS production, and increased glycolysis.

CONCLUSION: Transient exposure to commensal bacteria in early life modulates the host's immunity and protects from systemic bacterial infection in adulthood through long-lasting reprogramming of bone marrow precursor cells.

Keywords: Microbiota; Trained Immunity; Infection.

2930 - Category: INNATE IMMUNITY (IN)

Extracellular vesicles from neutrophils promote proinflammatory effects on peripheral blood mononuclear cells through NF-κB activation

SANTO, C.C.D.E.¹; DA SILVA, L.T.P.¹; GONÇALVES, B.S.¹; XAVIER, F.H.D.C.¹; DE MIRANDA, B.T.²; WACLAWIAK, I.³; COSTA, A.G.B.³; BLANCO, L.A.A.¹; TEMEROZO, J.R.¹; BOU-HABIB, D.C.¹. 1. LABORATORY ON THYMUS RESEARCH, OSWALDO CRUZ INSTITUTE/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. LABORATORY OF CELLULAR AND MOLECULAR CARDIOLOGY, CARLOS CHAGAS FILHO INSTITUTE OF BIOPHYSICS, UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. LABORATORY ON INNATE IMMUNITY, INSTITUTE OF MICROBIOLOGY PAULO DE GÓES, UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Extracellular vesicles (EVs) are small particles limited by lipidic bilayer membrane, secreted by different cell types to the extracellular environment, where they participate in cellular communication and regulate physiological functions. Neutrophil-derived EVs act as important mediators in inflammatory responses, however, the effects of neutrophil-derived EVs in peripheral blood mononuclear cells (PBMCs) are not completely clear. Here, we investigated whether neutrophil EVs promote immunomodulatory activity in human PBMCs. Neutrophils and PBMCs were obtained from buffy coats of healthy donors by density gradients, and EVs were isolated from neutrophil supernatant by differential ultracentrifugation. PBMCs were exposed to EVs (6.25-100 µg/mL) and, after 24h, the cytokine levels were measured in cell culture supernatant by ELISA. Cell viability was measured by LDH and XTT. To evaluate NF-κB activation, PBMCs were treated with EVs (50 µg/mL) and p65 phosphorylation was measured by ELISA. In some assays, EV-exposed PBMCs were pre-treated with a NF-κB inhibitor, followed by measurement of cytokine levels after 24h. The interaction of PBMCs subsets with EVs (100 µg/mL) was accessed by confocal microscopy and flow cytometry. Dot blot analysis detected specific EV markers CD63 and CD81, and nanoparticle tracking analysis showed that EV size was <160nm. EVs promoted the release of TNF-α, IL-6, IL-8 and IL-1β, but not IL-10, without affecting cellular viability. EVs promoted an increase of NF-κB p65 phosphorylation, whose inhibition strongly reduced the cytokine production. EVs did not carry detectable amounts of cytokines or relevant concentration of endotoxin (<0.181 EU/mL). We found that 80% of CD14+ cells interact with and internalize EVs, whereas only 1 - 1.5% of CD4+ and CD8+ interact with EVs. Together, our results suggest that neutrophil-derived EVs act as proinflammatory agents on PBMCs through NF-κB pathway.

Keywords: Neutrophil extracellular vesicles; Immunomodulatory effect; PBMCs.

2936 - Category: INNATE IMMUNITY (IN)

Effects of Polyethylene Terephthalate (PET) Microplastics on Macrophage Cell Line: An Experimental Analysis

DA SILVA, L.P.¹; MOREIRA, F.D.P.P.¹; DIAS, T.D.S.¹; BELARMINO, N.D.L.¹; COSENTINO-GOMES, D.¹; DE SOUSA FILHO, I.A.¹; FREIRE-DE-LIMA, C.G.²; DECOTE-RICARDO, D.¹; DA SILVA, L.H.P.¹. 1. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, SEROPÉDICA - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Microplastics (MPs) are pollutants derived from the degradation of plastics, commonly found in water and food packaging, as well as in synthetic fabrics. These residues are detected in water, soil, food, and in the bodies of both humans and animals. Macrophages are immune system cells that play a key role in innate immunity, acting as one of the first lines of defense between the internal and external environments. *Post-mortem* studies indicate the presence of MPs in various organs of humans and animals, such as gonads, lungs, liver and brain, and infer that the presence of these polymers causes harm to health. Thus, the aim of this study was to evaluate the effect of MPs on macrophages activities. For this purpose, MPs derived from PET bottles and murine macrophages RAW 264.7 cell line were used to investigate its effect. Initially, macrophage viability was assessed using the XTT assay, which showed that incubation with MPs reduced cell viability around 20 % in 24h. Next, the binding and phagocytosis capacity of macrophages were evaluated using *Saccharomyces cerevisiae* as a particle in the presence or not of MPs. The pretreatment of macrophages with MPs did not alter the initial binding of *S. cerevisiae* to macrophages, however the percentage of infected macrophages after 4 h of interaction increased 1.3-fold. The simultaneous incubation of macrophages with *S. cerevisiae* and MPs at 1 mg/mL augmented the yeast binding to macrophages in 1.3 fold, and increased macrophage phagocytosis in 1.5 fold compared to control. Additionally, preliminary results showed that in the presence of MPs [1 mg/mL] IFN- γ and LPS activated macrophages produced more nitric oxide compared to the control. As future perspectives, the reactive oxygen species (ROS) production by these cells upon exposure to MPs will be analyzed and the expression of pro-inflammatory and anti-inflammatory cytokines will be evaluated. Supported by: FAPERJ

Keywords: Microplastics;Macrophages;Innate immunity.

2939 - Category: INNATE IMMUNITY (IN)

Butyrate mitigates the worsening of oxidative stress and attenuates the cytokines secretion in human macrophages exposed to cigarette smoke and infected with SARS-CoV-2

DE CARVALHO, F.A.S.¹; ROSA, P.C.S.¹; DE OLIVEIRA, A.P.L.²; BITAR, R.³; CRUZ, E.F.³; SANTOS, R.A.³; JANINI, L.M.R.³; OLIMPIO, F.¹. 1. UNIFESP, SÃO JOSÉ DOS CAMPOS - SP - BRASIL; 2. UNINONE, SÃO PAULO - SP - BRASIL; 3. UNIFESP, SÃO PAULO - SP - BRASIL.

Background: Oxidative stress imbalance and pro-inflammatory cytokine storm are characteristic features observed in chronic obstructive pulmonary disease (COPD). Therefore, COPD individuals are indeed more susceptible to experiencing exacerbations triggered by lung infections, including COVID-19. Although the butyrate is a known anti-inflammatory in COPD, whether the butyrate acts on oxidative stress in macrophages exposed to cigarette smoke extract (CSE) and infected with SARS-CoV-2 remains unknown. **Objective:** Thus, the present study investigated the butyrate effect on oxidative stress and cytokine secretion in human macrophages exposed to CSE and SARS-CoV-2. **Methods:** The human macrophages were cultured, treated with butyrate, stimulated with CSE, and infected with SARS-CoV-2. The cellular viability was evaluated using trypan blue assay and cytotoxicity measured by lactate dehydrogenase (LDH) activity. The HO-1, NADPHoxidase, iNOS, Nrf2, and NF- κ B were quantified using specific PCR. The cytokines and chemokines were measured using specific ELISA kits. **Results:** Data show that butyrate decreased lipidic peroxidation, pro-oxidant metabolites and enzymes in human macrophages exposed to CSE and infected with SARS-CoV-2. On the other hand, SOD, catalase, GPx, HO-1 and Nrf2 were restored in human macrophages exposed to CSE and SARS-CoV-2 and treated with butyrate. Pro-inflammatory secretion was attenuated after treatment with butyrate. **Conclusion:** Therefore, butyrate attenuates oxidative stress unbalance and pro-inflammatory mediators' secretion in CSE-exposed human macrophages and infected with SARS-CoV-2, which suggests an action mechanism for butyrate against COPD exacerbation.

Keywords: COPD;SARS-CoV-2;human macrophages.

2946 - Category: INNATE IMMUNITY (IN)

Macrophage's metabolic rewiring induced by mycobacterial nonpolar lipids

DE ARAÚJO, L.E.¹; HIPÓLITO, C.B.G.D.A.¹; OLIVEIRA, Y.D.S.¹; CATALDI, T.R.²; LABATE, C.A.²; CANUTO, G.A.B.³; PETRILLI, J.D.¹; ARRUDA, S.¹; SILVA, A.Q.⁴. 1. INSTITUTO GONÇALO MONIZ, SALVADOR - BA - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, PIRACICABA - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 4. UT SOUTHWESTERN MEDICAL CENTER, DALLAS - ESTADOS UNIDOS DA AMERICA.

During an infection, *Mycobacterium tuberculosis* (Mtb) rearranges its cell wall lipids in response to the host-imposed stressful conditions. The mammalian cell entry (mce)1-4 operons encode mycobacterial transporters that mediate lipid uptake across the cell envelope. Changes in their expression alter the cell wall lipid composition and the pattern of inflammatory response induced by the mycobacteria. In previous investigations, we assessed the macrophage's pro-inflammatory response triggered by nonpolar lipid extracts harvested from Mtb strain disrupted in the mce1 operon (Δ mce1) and its parental wild-type (WT). Our findings suggest that Mtb lipids from Δ mce1 abrogate the level of pro-inflammatory markers triggered by WT lipids. Interestingly, WT's nonpolar lipids themselves induce an immunoregulatory state in macrophages, marked by increased expression of both M1/M2 markers, and raise the question whether mycobacterial lipids metabolically reprogram macrophages to support the M1 polarization. Here, GC-MS-based metabolomics analysis from WT lipids induced macrophages revealed increased levels of stearic and palmitic acids, indicating a shift in fatty acid biosynthesis. To assess whether nonpolar lipids alter the tricarboxylic acid (TCA) cycle functioning, we evaluated the expression of target genes in cells cultured with lipids from WT and Δ mce1 strains. WT lipids elicited a marked downregulation of *IDH* and upregulation of *ACLY*, *IRG1*, and *HIF1- α* , a metabolic program suggestive of early disruption of the TCA cycle and increased fatty acid synthesis. Δ mce1 lipids reduced the expression of *ACLY* and *HIF1- α* , relative to the WT-lipids stimulus. These findings suggest that the WT lipid-induced proinflammatory phenotype is supported by the metabolic rewiring of the TCA cycle and a shift of its metabolites to feed the fatty acid synthesis. Changes in the expression of Mce1 would abrogate the M1 polarization in favor of the alternative phenotype. Keywords: *Mycobacterium tuberculosis* lipids; Macrophage Polarization; Immunometabolism.

2955 - Category: INNATE IMMUNITY (IN)

Neutrophil Extracellular Traps Alter the Response of Human Peripheral Blood Mononuclear Cells and Monocyte-Derived Dendritic Cells

ALEXANDRE, S.C.D.S.; DA SILVA, J.O.; WACLAWIAK, I.; COELHO, S.V.A.; SARAIVA, E.; COSTA, A.G.B.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Neutrophils are the first immune cells recruited to inflammatory sites and can release neutrophil extracellular traps (NETs), web-like structures composed of DNA, histones, and granular proteins, under infectious or inflammatory conditions. NETs can capture and neutralize various pathogens, including parasites, bacteria, and viruses. Our group previously demonstrated that *Leishmania amazonensis* induces NET release and is susceptible to NET-mediated killing. Moreover, NETs have been detected in the blood of patients infected with Chikungunya virus (CHIKV). Beyond their antimicrobial activity, NETs also modulate immune responses. During *L. amazonensis* infection, monocytes can differentiate into dendritic cells (moDCs), which bridge the innate and adaptive immune systems by activating T cells. In CHIKV infection, monocytes and plasmacytoid dendritic cells within peripheral blood mononuclear cells (PBMCs) play key roles in initiating cytotoxic responses. This study investigated the immunomodulatory effects of NETs induced by *L. amazonensis* on moDCs infected or not with the parasite, and of PMA-induced NETs on PBMCs infected or not with CHIKV. PBMCs and monocytes from healthy donors were used to generate moDCs (CD1a+). Both cell types were infected and then treated with NETs to evaluate activation and cytokine responses. We found that NETs modulate cytokine production in both moDCs and PBMCs. NET treatment increased IL-8 release by moDCs, promoting neutrophil migration. However, HLA-DR and CD86 expression remained unchanged. In PBMCs, NETs enhanced MCP-1 production in response to CHIKV, while IFN- α levels were reduced. In conclusion, our results suggest that NETs influence the cytokine milieu of innate immune cells, potentially shaping local inflammatory responses during parasitic and viral infections.

Keywords: 1: Neutrophil extracellular traps; Dendritic cells; Peripheral Blood Mononuclear Cells.

2970 - Category: INNATE IMMUNITY (IN)

Nucleic Acid Sensing Pathways in a Murine Model of Hypersensitivity Pneumonitis

DE AZEVEDO, Y.G.; DE ALMEIDA, V.M.; HERRERA, A.J.R.; FABRO, A.T.; ALVES-FILHO, J.C.; RITEAU, N.. USP, RIBEIRÃO PRETO - SP - BRASIL.

Hypersensitivity pneumonitis (HP) is the third most prevalent interstitial lung disease worldwide, triggered by repeated exposure to inhaled antigens such as organic dust, molds, bacteria, or avian proteins present in occupational or environmental settings. This condition is characterized by an exacerbated immune response primarily driven by T lymphocytes, which may progress to chronic inflammation and pulmonary fibrosis. Despite its clinical relevance, the molecular mechanisms underlying HP remain largely unexplored, limiting the development of effective therapies. However, as previously shown, chronic inflammation and tissue damage in HP may lead to the release of host-derived nucleic acids, which in turn can activate innate immune sensors. Among these, the STimulator of Interferon Genes (STING) has emerged as a key regulator in HP pathophysiology. Based on this, in this study, we aim to investigate the role of nucleic acid sensing pathways, particularly STING, in HP. To assess this, we are implementing a murine model of HP by oropharyngeal instillation of inactivated *Saccharopolyspora rectivirgula*, a clinically relevant antigen that mimics occupational exposure in HP. Preliminary data revealed peribronchial infiltrates with lymphoid aggregates. Flow cytometry of the pulmonary tissue indicated increased frequencies of CD4⁺ T cells, neutrophils and alveolar macrophages. These findings support the establishment of an important inflammatory response in the model. Therefore, this study opens perspectives for identifying the relevance of nucleic acid sensing pathways and for identifying novel immunopathogenic mechanisms and potential therapeutic targets in HP. Importantly, clinical samples will be analyzed for instance by spatial transcriptomics to assess our results in human HP.

Keywords: Hypersensitivity pneumonitis;Nucleic acid sensing;STING.

2975 - Category: INNATE IMMUNITY (IN)

Exploring the role of the mechanosensitive ion channel Piezo1 in macrophages in an experimental model of PsoriasisDE OLIVEIRA, M.F.¹; BOS, N.N.²; DE OLIVEIRA, T.G.³; LEBANI, P.L.²; POLIDORO, J.Z.²; GONÇALVES, L.E.D.⁴; CAMARA, N.O.S.². 1. UNIVERSITY OF SÃO PAULO - BRAZIL, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. – FRIEDRICH-ALEXANDER UNIVERSITY ERLANGEN-NÜRNBERG, ERLANGER - ALEMANHA.

Psoriasis is a chronic, immune-mediated inflammatory skin disease affecting ~1–3% of the global population. It primarily affects the skin and joints, as in psoriatic arthritis, and is marked by epidermal hyperproliferation and cytokine imbalance, particularly involving the interleukin (IL)-23/IL-17 axis. Classically activated (M1-polarized) macrophages play a key role in sustaining inflammation. This study investigates the role of the mechanosensitive ion channel Piezo1 in macrophages and keratinocytes in psoriasis. In macrophages, Piezo1 activates pro-inflammatory pathways such as nuclear factor kappa B (NF-κB), promoting the M1 phenotype. In keratinocytes, it regulates proliferation, migration, and epithelial-mesenchymal transition through calcium (Ca²⁺) influx, aiding tissue regeneration and remodeling. A murine model of imiquimod-induced psoriasis was used, with topical treatment using a Piezo1 agonist (Yoda1) and antagonist (Dooku1), along with histological analysis, cytokine and chemokine profiling, protein expression, and inflammatory infiltrate characterization. We hypothesized that Piezo1 inhibition would reduce inflammation and cell proliferation in lesions. Initial results showed increased Piezo1 in macrophages and endothelial cells in psoriatic skin. Myeloid-specific Piezo1 knockout led to a gene expression profile indicating a less pro-inflammatory phenotype. In vitro, Piezo1 activation promoted keratinocyte proliferation up to a threshold, while inhibition reduced it. Psoriasis induction in vivo caused strong skin inflammation, M1 macrophage and neutrophil infiltration, and marked epidermal hyperproliferation. Metabolic analysis revealed increased glycolysis in inflammatory macrophages and S100A8/A9 protein overexpression, reinforcing the inflammatory loop. These findings suggest Piezo1 integrates mechanical, metabolic, and immune signals and is a potential therapeutic target for modulating the immune-epithelial microenvironment in psoriasis.

Keywords: Yoda1;Dooku1;Skin.

2981 - Category: INNATE IMMUNITY (IN)

Atroxlysin-I (Atr-I) and Atroxlysin-III (Atr-III) metalloproteinases from Bothrops atrox snake venom alter cytokine production and activate signaling pathways

PARREIRAS, L.G.¹; BARBOSA, M.P.¹; DE ALVARENGA, V.G.²; DE QUEIROZ, N.M.G.P.³; RUIZ, D.E.V.⁴; HORTA, M.D.F.M.¹; SILVA, L.M.²; SANCHEZ, E.O.F.²; DE OLIVEIRA, L.S.². 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. FUNDAÇÃO EZEQUIEL DIAS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. UNIVERSIDAD NACIONAL MAYOR DE SAN MARCOS, LIMA - PERU.

Atroxlysin-I (Atr-I) and Atroxlysin-III (Atr-III) are snake venom metalloproteinases (SVMPs) isolated from Peruvian *Bothrops atrox*. Snake venoms are composed of a complex mixture of proteins with diverse bioactive functions, with SVMPs being the most abundant in the *B. atrox* proteome. The pathophysiology of snakebite envenomation includes hemorrhage caused by cleavage of basement membrane components, exacerbated inflammation, necrosis, and, in severe cases, limb loss. Atr-I and Atr-III have been previously characterized as hemorrhagic, fibrinolytic, and inhibitors of platelet aggregation. However, little is known about their role in the innate inflammatory response induced during envenomation. In this study, we evaluated the effects of Atr-I and Atr-III on the innate immune system and related signaling pathways using bone marrow-derived macrophages (BMDMs) from C57BL/6 mice (CEUA 011/2020). SVMPs differentially modulate the mRNA expression of inflammatory cytokines. Atr-I strongly induces IL-6 (620-fold) and TNF- α (63-fold), while Atr-III more efficiently increases IL-1 β expression (225-fold). However, only IL-1 β was detected in the supernatant of macrophages stimulated with both SVMPs. These proteins also negatively modulate cytokine production in macrophages previously stimulated with Pam₃CSK₄ (TLR2) and ultrapure LPS (TLR4). A reduction in cytokine levels was observed due to differential proteolytic cleavage: Atr-I cleaves IL-6 and TNF- α , while Atr-III cleaves TNF- α and IL-1 β . Additionally, Atr-I activates ERK1/2 and p38 pathways, which are involved in stress signaling, inflammation, and cell proliferation. These findings suggest that SVMPs may be recognized as molecular patterns, activating signaling pathways and interfering with cytokine production by macrophages. Therefore, this study contributes to the characterization of the biological activity of Atr-I and Atr-III and to the understanding of the inflammatory response during envenomation.

Keywords: snake venom metalloproteinases (SVMPs); macrophage; cytokines.

2990 - Category: INNATE IMMUNITY (IN)

Secretion of proteases by *Leptospira*, cleavage of Complement proteins and effect on phagocytosis

DOS SANTOS, L.B.R.¹; AMAMURA, T.A.¹; ISAAC, L.¹; HEINEMANN, M.B.²; COURROL, D.D.S.³; BARBOSA, A.S.³. 1. INSTITUTO DE CIÊNCIAS BIOMÉDICAS, SÃO PAULO - SP - BRASIL; 2. FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA, SÃO PAULO - SP - BRASIL; 3. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

Leptospirosis is a neglected zoonotic disease caused by spirochetes of the genus *Leptospira*. With a global distribution, it is especially prevalent in tropical regions and socially vulnerable populations. The infection ranges from subclinical to severe presentations, and the ability of pathogenic leptospires to colonize the host is largely linked to immune evasion strategies. Among these, the secretion of bacterial proteases capable of degrading key components of the innate immune response, such as the Complement System, plays a central role.

In this study, we investigated the *in vitro* effects of secreted products from pathogenic *Leptospira* species and the purified recombinant protease thermolysin on Complement proteins and on human phagocytic cells. Culture supernatants from *L. interrogans* strains L1 130 and Canicola, as well as recombinant thermolysin, were able to cleave the human Complement proteins C3, C3b and iC3b in a dose- and time-dependent manner. In parallel, THP-1 cells exposed to these secreted products showed significantly impaired phagocytosis of zymosan particles, especially at higher concentrations of L1 130 culture supernatants and thermolysin. In contrast, secreted products from the saprophytic strain *L. biflexa* Patoc had no detectable effect on Complement cleavage or phagocytic capacity.

Flow cytometry analysis revealed no significant changes in the expression of surface receptors TLR2, Fc γ R1, CR3 or CR4 on THP-1 macrophages following treatment with *Leptospira* secreted fractions, indicating that the observed effects on phagocytosis were primarily due to degradation of soluble opsonins rather than receptor modulation. Together, these findings support the role of leptospiral proteases as immune invasion factors capable of modulating key innate immune mechanisms.

Keywords: *Leptospira*; Proteases; Complement System.

2993 - Category: INNATE IMMUNITY (IN)

METALLOPROTEINASES AND PHOSPHOLIPASE A₂ ISOLATED FROM BOTHROPS LEUCURUS VENOM MODULATE MACROPHAGE ACTIVATION

BARBOSA, M.P.¹; PARREIRAS, L.G.¹; DE ALVARENGA, V.G.²; SILVA, L.M.²; SANCHEZ, E.O.F.²; HORTA, M.D.F.M.¹; DE OLIVEIRA, L.S.². 1. FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. EZEQUIEL DIAS FOUNDATION, BELO HORIZONTE - MG - BRASIL.

Snakebite envenoming is considered a neglected tropical disease with high mortality and morbidity rates. Bites by *Bothrops* can cause a pronounced inflammatory response, leading to classic manifestations including pain, edema, endothelial damage, necrosis at the bite site, and leukocyte migration. These symptoms are associated with the venom components, notably phospholipases A₂ (PLA₂), L-amino acid oxidase, serine proteinases (SVSPs), and metalloproteinases (SVMPs), being described primarily in the *Bothrops leucurus* species. Although the role of these toxins in the inflammatory response is partially known, a complete understanding of their immunobiological properties and their interaction with the innate immune system remain unclear. Therefore, this study aims to understand the effects of SVMPs and PLA₂ on macrophages. To verify whether these toxins could modulate macrophage activation, RAW 264.7 cells were prestimulated with Pam₃CSK₄ or LPS, which are agonists of Toll-like receptors (TLRs) 2 and 4, respectively, and then treated with Leuc-a, Leuc-B, and PLA₂. After 24 hours of cell culture with these treatments, the supernatants were collected for IL-6, IL-1 β , and TNF- α quantification by ELISA. All the toxins evaluated reduced TNF- α production induced by LPS and Pam₃CSK₄. Additionally, Leuc-a reduced IL-6 production induced by TLR 2 and 4 agonists. Leuc-B reduced IL-6 production induced by LPS while increased IL-6 production induced by Pam₃CSK₄. The toxins induced a slight increase in IL-1 β production when macrophages were pretreated with Pam₃CSK₄ or LPS. Analysis of the enzymatic activity of SVMPs demonstrated the ability of Leuc-a and Leuc-B to cleave the cytokine TNF- α and Leuc-B to cleave IL-6. Furthermore, preliminary results demonstrated that the toxins are internalized by macrophages, and SVMPs induce phosphorylation of ERK1/2. In conclusion, our results suggest that SVMPs and PLA₂ are recognized by macrophages and modulate cytokine production.

Keywords: snake venom proteins;inflammation;macrophage.

3009 - Category: INNATE IMMUNITY (IN)

Neutrophil Extracellular Traps: Unraveling Molecular Signatures Through Proteomic Profiling

TEMEROZO, J.¹; GONÇALVES, B.S.²; SABOIA-VAHIA, L.³; FORMIGA JUNIOR, M.A.¹; PINHO, N.³; ROCHAEL, N.C.¹; QUESNE, A.H.M.L.¹; SARAIVA, E.⁴; ESCOBAR, P.C.³; BOU-HABIB, D.C.¹. 1. LABORATORY ON THYMUS RESEARCH, OSWALDO CRUZ INSTITUTE/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. LABORATORY ON THYMUS RESEARCH, CRUZ INSTITUTE/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. LABORATORY ON LEISHMANIASIS RESEARCH, OSWALDO CRUZ INSTITUTE/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 4. LABORATORY OF IMMUNOBIOLOGY OF LEISHMANIASIS, UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Neutrophil Extracellular Traps (NETs) are intricate, web-like structures primarily composed of decondensed chromatin, histones, and a diverse array of granular and cytoplasmic proteins. Released by activated neutrophils, these extracellular networks serve as a critical component of the innate immune response, acting as a physical barrier to entrap and neutralize invading pathogens. Beyond direct microbial killing, NETs have emerged as multifaceted players in modulating inflammation, participating in cellular communication, and in the regulation of tissue homeostasis, or dysregulation thereof, in various physiological and pathological conditions. The characterization of its molecular composition is critical to the understanding of its role in innate immunity, cellular communication, and tissue homeostasis. Also, NET protein composition in different disease states could serve as valuable biomarkers for diagnosis, prognosis, or monitoring therapeutic responses. Through a proteomic approach we identified up to 2500 proteins present in NETs obtained with several stimuli (basal liberation, IL-8, HIV-1, SARS-CoV-2 and *Leishmania amazonensis*), with several differentially expressed between them. Enrichment analysis showed stimuli-specific profile distribution of ontologies (subcellular location, molecular function and biological processes). Such data set reinforces the complexity of NETs composition giving the possibility to identify signature markers and better understand their relationship with the biological actions of NETs. This detailed molecular insight is essential for harnessing the beneficial aspects of NETs while mitigating their detrimental effects in various inflammatory and autoimmune conditions.

Keywords: NETs;Proteomics;Neutrophils.

3018 - Category: INNATE IMMUNITY (IN)

Human Monocyte Exhaustion Is Associated With Upregulation Of The Cellular Senescence Pathway

DAMAS, M.S.F.; DE LIMA, J.O.; FRACAZZO, M.C.C.; CASTRO, R.; SUGIYAMA, F.H.C.; FONTANARI, C.; FRANTZ, F.G.. UNIVERSIDADE DE SÃO PAULO - FACULDADE DE CIÊNCIAS FARMACÊUTICAS, RIBEIRÃO PRETO - SP - BRASIL.

Introduction: Monocyte exhaustion is associated with metabolic reprogramming and mitochondrial functional impairment. Mitochondrial dysfunction is often linked to early cellular senescence. Characterization of human monocyte exhaustion and cellular senescence is poorly described in the literature. **Objectives:** This study aims to investigate the expression of specific markers to characterize the mechanisms underlying monocyte exhaustion. **Methods:** Monocytes were isolated from healthy donors (n=6) at the Hemocentro de Ribeirão Preto and monocyte exhaustion was induced *in vitro*. Membrane protein expression of exhaustion marker was evaluated through flow cytometry. UMAPs were generated using FlowJo v10.10. Gene expression of exhaustion markers was assessed via RT-qPCR. Cytokine production was quantified using ELISA. **Results:** We observed that exhausted monocytes presented upregulation of several senescence associated genes, with sustained SASP-associated cytokine expression. UMAP analysis identified 5 distinct clusters in which 2 clusters were enriched in exhausted monocytes. These clusters correlated differently to genes associated with regular immune response and cellular senescence. **Conclusions:** Together, our data suggests that monocyte exhaustion and cellular senescence are connected. Further studies are necessary to explore the metabolic pathways connecting these two phenotypes.

Keywords: Monocyte;Cellular exhaustion;Cellular senescence.

3049 - Category: INNATE IMMUNITY (IN)

Role of the NLRP3 inflammasome in the formation of DNA extracellular traps by monocytes in response to SARS-CoV-2 and its spike protein

DA SILVA, G.C.P.¹; TEMEROZO, J.²; ROCHAEL, N.C.²; ROZINI, S.V.²; GONCALVES, R.M.²; BOZZA, P.T.²; BOU-HABIB, D.C.²; SARAIVA, E.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

It is already known that SARS-CoV-2 virus triggers the release of Neutrophil Extracellular Traps (NETs), which are associated with the severity of COVID-19. Monocytes also release DNA Extracellular Traps (DETs), analogous to NETs, in response to various stimuli. It has been demonstrated that the SARS-CoV-2 virus and its spike protein induce the formation of the NLRP3 inflammasome in monocytes, resulting in ASC recruitment, caspase-1 activation, and the release of IL-1 β /IL-18, accompanied by pyroptosis via GSDMD (gasdermin D). Activation of the NLRP3 inflammasome can promote hyperinflammation, pulmonary and systemic tissue damage, and trigger uncontrolled immune responses, which can be potentially lethal in COVID-19. Here, we demonstrate that human peripheral blood monocytes stimulated by beta-propiolactone-inactivated SARS-CoV-2 (inSARS-CoV-2) and its spike protein release DETs with a characteristic morphology, showing DNA associated with elastase, myeloperoxidase, and H3 citrullinated histone. Using specific inhibitors, we demonstrated that inSARS-CoV-2-induced DETs in monocytes depend on the interaction of the virus with TLR2 and TLR4 receptors. We also showed that inhibiting NLRP3, caspase, and GSDMD reduced the extrusion of DETs induced by inSARS-CoV-2 and the spike protein. Furthermore, there was an increase in LDH and IL-1 β release by monocytes activated by inSARS-CoV-2 and its spike protein. Through immunofluorescence, we showed that DETs induced by the virus and its spike protein contain ASC speck, NLRP3, cleaved caspase-1, and IL-1 β . Our findings suggest that inflammasome activation is involved in the release of DET by monocytes.

Keywords: Monocytes;SARS-CoV-2;NLRP3.

3066 - Category: INNATE IMMUNITY (IN)

A Natural Microbiome Species Induces Heritable Defense Against Intracellular Pathogens in the Intestinal Epithelium

LAZETIC, V.; WEST, J.D.; SCHWARTZ, S.; LI, S.; ROPER, P. THE GEORGE WASHINGTON UNIVERSITY, WASHINGTON - ESTADOS UNIDOS DA AMERICA.

The microbiome shapes host immunity, yet the molecular cues by which specific bacteria modulate innate defenses are not fully understood. The nematode *Caenorhabditis elegans*, a genetically tractable model organism with an innate immune system and a defined microbiome, provides a powerful system for studying these interactions. One of the key defense pathways in *C. elegans* is the Intracellular Pathogen Response (IPR), a transcriptional program activated by viral and fungal obligate intracellular pathogens of the intestine, which shares similarities with the mammalian type I interferon response. To identify microbiome-derived IPR triggers, we screened 12 native bacterial isolates for their ability to activate an IPR fluorescent reporter and found that *Stenotrophomonas indicatrix* (JUb19) strongly induces reporter expression in the intestine, epidermis, neurons, and somatic gonad. In addition to JUb19, other *Stenotrophomonas* species can also activate this response, indicating it may be a genus-conserved feature. Unlike previously described IPR triggers, JUb19 resides extracellularly in the intestinal lumen, making it the first non-invasive microbe known to activate this response. Heat-killed JUb19 fails to induce reporter expression, whereas mechanically or chemically inactivated bacteria retain this activity, pointing to a heat-sensitive molecular trigger. Preliminary evidence suggests that bacterial RNA may contribute to IPR activation. Transcriptomic analyses revealed that JUb19 induces a subset of IPR and lysozyme genes, enhancing host resistance to intracellular pathogens. Notably, this protective effect is also observed in the progeny. However, JUb19 exposure reduces fitness, highlighting a trade-off between immunity and development. Together, these findings identify a novel extracellular mechanism of microbiome-induced epithelial immunity in *C. elegans*.

Keywords: Microbiome;Inherited immunity;Intracellular pathogens.

3071 - Category: INNATE IMMUNITY (IN)

Modulation of innate immune response by Aedes aegypti salivary proteins during Chikungunya virus infectionDA SILVA, J.O.¹; WACLAWIAK, I.¹; MATTOS, T.R.F.¹; COELHO, S.V.A.¹; OLIVEIRA, G.D.N.¹; GONÇALVES, M.¹; OLIVEIRA, F.²; COSTA, A.G.B.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. NATIONAL INSTITUTES OF HEALTH, BETHESDA - ESTADOS UNIDOS DA AMERICA.

Chikungunya virus (CHIKV) is transmitted when the Aedes mosquito takes a blood meal, injecting both the virus and its salivary proteins into the host. Few studies have demonstrated the mechanisms involved in the modulation of immune responses induced by specific Aedes saliva proteins during viral infections. We hypothesize that the salivary proteins AED7L2 and apyrase, which are abundant in Aedes aegypti saliva, influence innate immune cell responses and impact CHIKV infection. To test this, we infected the ears of C57BL/6 mice with purified CHIKV and analyzed immune cell infiltration in the ear and draining lymph nodes (dLNs) 16 hours post-infection. Our data show that both AED7L2 and apyrase alter the immune cell profile compared to CHIKV infection alone. In the ear, AED7L2 and apyrase increased the percentage of F4/80+ macrophages and neutrophils. Specifically, AED7L2 enhanced the expression of activation markers CD43 and CD206 on macrophages and induced early recruitment of intermediate (CD43⁺Ly6C^{int}CD11b⁺) and non-classical (CD43⁺Ly6C⁺CD11b⁺) monocytes to the infection site. In the dLNs, AED7L2 decreased the percentage of F4/80+ macrophages and neutrophils, while apyrase increased their presence. Furthermore, AED7L2 reduced the expression of CD86, MHC II, and CD43 on macrophages in dLNs, whereas apyrase had the opposite effect. Since we observed differences in the number of macrophages we evaluated if these proteins would influence CHIKV in vitro infection. Treatment of macrophages with AED7L2 reduced the amount of active viral particles quantified by plaque assay, while apyrase had no impact on virus infection. Our results indicate that AED7L2 and apyrase modulate the host immune response and suggest that these salivary proteins could play a significant role in the pathogenesis of chikungunya virus infection.

Keywords: Chikungunya virus;salivary proteins;Aedes.

3072 - Category: INNATE IMMUNITY (IN)

Characterization of mast cell activation by PAMPs and different morphologic forms of *Aspergillus fumigatus*

DE LIMA, B.C.¹; DE ARAUJO, W.G.¹; DETONI, M.A.A.²; FIGUEIREDO, R.T.²; DIAZ, B.L.¹. 1. INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO (IBCCF/UFRJ), RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO DE CIÊNCIAS BIOMÉDICAS (ICB/UFRJ), RIO DE JANEIRO - RJ - BRASIL.

Aspergillus fumigatus is an airborne opportunistic fungus that can cause severe pulmonary diseases, particularly in immunocompromised or hypersensitive individuals. Among its structural components, chitin and β -glucan are key Pathogen-Associated Molecular Patterns (PAMPs), capable of modulating immune responses in the lung. Mast cells (MCs) are innate immune effector cells involved in allergy, infection, and chronic inflammation, but their role in antifungal defense remains poorly characterized. In particular, it is unclear whether MCs contribute to the inflammatory response following exposure to *A. fumigatus*.

This study aims to characterize the innate response of MCs to *A. fumigatus* and its structural PAMPs, β -glucan and chitin. We used murine bone marrow-derived MC precursors immortalized by inducible overexpression of Hoxb8 (iMCps) cultivated with IL-3 (2 ng/mL). Removal of tamoxifen for 6-8 days differentiated the immortalized cells into MCs (iMCds) with maturation markers: metachromatic granules; surface c-kit and Fc ϵ RI expression. Cells were stimulated with *A. fumigatus* conidia and hyphae, purified chitin and β -glucan, and evaluated for degranulation, cytokine and inflammatory mediator release, and reactive oxygen species (ROS) production.

Stimulation with *A. fumigatus* conidia and hyphae induced degranulation of iMCds, but not in precursors. Conidia also led to increased cysteinyl leukotriene (LTcys) production in iMCds only. In contrast, hyphae and chitin increased prostaglandin D2 (PGD2) production in iMCps but not in iMCds. *A. fumigatus* also stimulated IL-6 production in iMCds. These findings suggest that although *A. fumigatus* activates MCs, responses vary depending on cell maturation stage and fungal form. Further studies are needed to better understand how MCs respond to *A. fumigatus* and to identify which fungal components act as immunogenic triggers in this context.

Keywords: Mast cell; *Aspergillus*; Fungi.

3084 - Category: INNATE IMMUNITY (IN)

Putative role of VDAC2 as a component of non-canonical autophagy

ALVES, C.B.D.A.; FREITAS FILHO, E.G.; CUNHA, L.D.. FMRP-USP, RIBEIRÃO PRETO - SP - BRASIL.

During phagocytosis induced by the activation of certain receptors, conjugation of autophagic ATG8 family proteins, such as LC3, can occur on the single membrane of the phagosome - a process known as LC3-associated phagocytosis (LAP). LAP modulates macrophage responses to stimuli such as pathogens, immune complexes, and dead cells. The protein Rubicon (RUBCN) plays an essential role in LAP, being associated with the generation of phosphatidylinositol 3-phosphate (PI3P) and the production of intraphagosomal reactive oxygen species (ROS), two key events in this pathway. Previously, we identified VDAC2 (Voltage-dependent anion channel 2), an anionic channel located in the outer mitochondrial membrane, as a RUBCN-interacting protein through a proximity-dependent biotinylation (Turbo-ID) proteomic screen. Here, we aim to determine the importance of VDAC2 in LAP. We first established and characterized a system of VDAC2 depletion by CRISPR in primary (BMDM) and immortalized (iBMDM) bone marrow-derived macrophages derived of Cas9-expressing mice. We confirmed that VDAC2 depletion was specific, without affecting VDAC1 and VDAC3 protein levels. Basal levels of mitochondrial superoxide, measured with the MitoSOX probe, were similar between *Vdac2*^{-/-} and control BMDM, suggesting that mitochondrial oxidative status is preserved. High-resolution microscopy imaging of BMDM treated with opsonized-zymozan (Op-Zym), a stimulus that induces LAP, revealed the presence of VDAC2 on phagosomes. VDAC2 recruitment to phagosomes requires RUBCN, as its level were reduced in *Rubcn*^{-/-} iBMDM. LC3 lipidation on phagosomes containing Op-Zym was impaired in *Vdac2*^{-/-} BMDM and iBMDM, as assessed by confocal microscopy. Further, purification of phagosomes of BMDM stimulated with agarose beads coated with IgG indicate less recruitment of LAP components in the absence of VDAC2. Our data so far indicates that VDAC2 is a component of LAP.

Keywords: non-canonical autophagy; VDAC2; Rubicon.

3091 - Category: INNATE IMMUNITY (IN)

The role of Myosin IXB (Myo9b) in inflammasome activity

SOUZA, K.B.D.S.; CAMARGO, F.E.C.; PONTILLO, A.; DE ALENCAR, B.C.. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

The inflammasome is an intracellular protein complex activated by disturbances in homeostasis, resulting in the release of pro-inflammatory cytokines and pyroptosis. The cytoskeleton has emerged as a key element in inflammasome assembly and activation. Myosin IXB (Myo9b), an unconventional myosin with RhoGAP domain, regulates actin remodeling by inhibition of RhoA. Altered Myo9b expression has been linked to inflammatory diseases, but its role in inflammasome activation remains unclear. This study aims to investigate Myo9b's role in inflammasome modulation. Two Myo9b single nucleotide variants (rs1545620 and rs962917) were selected from the GTEx database and genotyped in healthy donor samples. IL-1 β production was assessed after inflammasome activation, revealing genotype-associated differences in monocyte-derived dendritic cells (MoDCs) stimulated via NLRP3. Based on these results and the known link between RhoA and pyrin inflammasome regulation, we also tested pyrin activation in MoDCs in addition to NLRP3. Inflammasome activation protocols were optimized, and lentiviral transduction controls were tested. Although initial tests showed IL-1 β production, subsequent assays revealed inconsistent responses, mainly a lack of IL-1 β production after pyrin stimulation. Gene expression analysis showed low MEFV levels in MoDCs, suggesting limited pyrin inflammasome expression. Given these findings, we focused on NLRP3 activation. MoDCs were transduced with lentiviral vectors expressing shRNAs targeting MYO9B and knockdown efficiency was confirmed by qPCR. IL-1 β production after NLRP3 stimulation was observed in all conditions, with no significant differences between silenced and control cells. Results showed high variability, and further experiments are needed to clarify the impact of Myo9b on inflammasome activity. Altogether, our findings highlight the complexity of inflammasome regulation and the need for robust models to evaluate cytoskeletal regulators such as Myo9b.

Keywords: Cytoskeleton;Dendritic Cell;RhoGTPase.

3109 - Category: INNATE IMMUNITY (IN)

Manilkara huberi Attenuates Experimental Colitis: Anti-Inflammatory and Antioxidant Effects in a DSS-Induced Murine Model

LOREDO, I.C.¹; DOS SANTOS, A.C.C.¹; VANZAN, D.F.²; DE OLIVEIRA, R.P.³; ARRUDA, A.C.⁴; ARRUDA, M.S.P.⁴; PYRRHO, A.D.S.²; MACHADO, J.C.⁵; FERNANDES, J.⁶; MADI, K.⁷; DE SOUZA, H.S.P.⁸; BENTES, J.¹; CASTELO-BRANCO, M.T.L.¹. 1. ICB; HUCFF, RIO DE JANEIRO - RJ - BRASIL; 2. SCHOOL OF PHARMACY; UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. ALBERTO LUIZ COIMBRA INSTITUTE FOR GRADUATE STUDIES AND ENGINEERING RESEARCH; UFRJ, RIO DE JANEIRO - RJ - BRASIL; 4. UFPA, PARÁ - PA - BRASIL; 5. ALBERTO LUIZ COIMBRA INSTITUTE FOR GRADUATE STUDIES AND ENGINEERING RESEARCH; COPPE; UFRJ, RIO DE JANEIRO - RJ - BRASIL; 6. MULTIDISCIPLINARY RESEARCH CENTER IN BIOLOGY; UFRJ, RIO DE JANEIRO - RJ - BRASIL; 7. HUCFF, RIO DE JANEIRO - RJ - BRASIL; 8. HUCFF; UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Manilkara huberi, an Amazonian endemic tree of the Sapotaceae family, has been traditionally used to treat various illnesses. Its genus is known for antiparasitic, antioxidant, anticancer, and particularly anti-inflammatory properties. Ulcerative colitis (UC) is an idiopathic, chronic inflammatory bowel disease restricted to the gastrointestinal mucosa. This study aimed to evaluate the anti-inflammatory and antioxidant effects of a methanolic extract of *M. huberi* (MH1) in a DSS-induced experimental colitis model. C57BL/6 mice received 3.0% DSS in drinking water for 7 days and were treated with three oral doses of MH1 (0.3 mg/kg) administered on alternate days. On day six, colonoscopy was performed (CEUA 080/22). Disease activity was evaluated using video-colonoscopy with ultrasonic endoluminal bioluminescence (UEB), histopathological scoring, ELISA cytokine profiling, Griess assay for nitric oxide, and tissue analysis via histology, immunohistochemistry, and immunofluorescence. MH1-treated mice showed reduced weight loss compared to DSS-only controls, despite unchanged colon length. Endoscopic and histological assessments confirmed that MH1 attenuated DSS-induced mucosal damage, ulceration, bleeding, and thickening. Treatment also led to increased TGF- β expression and normalization of key inflammatory pathways (ERK, NF- κ B, NLRP3, CASP1). Additionally, MH1 significantly lowered nitrite levels, indicating antioxidant activity. These findings indicate that *M. huberi* exerts both anti-inflammatory and antioxidant actions in experimental colitis, potentially through modulation of inflammatory signaling and oxidative stress. Investigation into *M. huberi* extracts and their isolated fractions may contribute to the development of novel plant-based therapeutics for inflammatory diseases.

Keywords: Manilkara huberi;Experimental colitis;Inflammation.

3115 - Category: INNATE IMMUNITY (IN)

Type III interferons drive intestinal pathology caused by loss of ADAR1 function

ROCHA, M.F.¹; DIERICK, E.¹; HESSMANN, E.¹; GONÇALVES, A.²; DIVERT, T.¹; LEMEIRE, K.²; XIE, W.¹; VANDENABEELE, P.¹; MAELFAIT, J.¹. 1. VIB-UGENT CENTER FOR INFLAMMATION RESEARCH / DEPARTMENT OF BIOMEDICAL MOLECULAR BIOLOGY, GHENT UNIVERSITY, GHENT - BELGICA; 2. VIB-UGENT CENTER FOR INFLAMMATION RESEARCH, GHENT - BELGICA.

Type I and III interferons (IFNs) are essential cytokines in antiviral defence. Chronic IFN production underlies the development of "type I interferonopathies". As the name suggests, the aetiology of these diseases has been attributed to chronic type I IFN overproduction. To study the potential contributions of type I or type III IFNs to the development of IFN-mediated disease, we developed an ADAR1 mutant mouse model (*Adar^{mutZa}*) that mimics the prototypical human type I interferonopathy Aicardi-Goutières Syndrome (AGS). Our data shows that while type I IFN signalling causes anaemia in *Adar^{mutZa}* mice, type III IFNs drive intestinal inflammation, characterized by intestinal cell death. Genetic removal of either the type I IFN receptor or type III IFN receptor only marginally extended the lifespan of *Adar^{mutZa}* mice, which died within 1 day after birth. However, *Adar^{mutZa}* mice which are unable to respond to both type I and III IFNs aged normally, were fertile and did not develop anemia or intestinal inflammation. To better understand the mechanisms of type III-mediated pathology, we developed a conditional *Adar-p150* knockout mouse line. Using a tamoxifen inducible whole body knockout approach, we show that removal of ADAR1-p150 in adult mice results in elevated mRNA expression levels of type I and III IFN in the intestine, along with the development of an interferon-stimulated gene (ISG) signature and intestinal cell death. Furthermore, intestinal epithelial cell-specific *Adar-p150* knockout mice die during development and develop an ISG response in intestinal epithelial cells. Lethality is rescued by genetic removal of the type III IFN receptor, which is in line with our data showing that type III IFNs cause intestinal pathology in *Adar^{mutZa}* mice. Together, this shows that type III IFNs contribute to intestinal pathology in a mouse model of AGS caused by ADAR1 loss of function.

Keywords: Type III interferon;Autoinflammation;Intestinal pathology.

3130 - Category: INNATE IMMUNITY (IN)

NLRP3 Inflammasome Activation in Endothelial Cells Drives Lung Inflammation in Coronavirus Infection

RODRIGUES, T.S.¹; PEREIRA, K.A.S.²; LOPES, F.²; HERRERA, A.J.R.²; BATAH, S.²; JÚNIOR, R.B.M.²; FABRO, A.T.²; COSTA, V.V.³; LEITE, J.A.⁴; ZAMBONI, D.S.². 1. UNIVERSITY OF SANTA CATARINA (UFSC), FLORIANÓPOLIS - SC - BRASIL; 2. USP, RIBEIRÃO PRETO - SP - BRASIL; 3. UFMG, BELO HORIZONTE - MG - BRASIL; 4. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Investigating inflammasome activation in endothelial cells infected with coronavirus is crucial for understanding COVID-19 pathogenesis and outcomes. Inflammasome activation may drive a dysregulated immune response, exacerbating inflammation, endothelial damage, and thrombosis. Understanding these mechanisms is essential for developing targeted therapies to mitigate severe COVID-19 and improve clinical outcomes. **Aim:** To investigate NLRP3 inflammasome activation in endothelial cells during coronavirus infection and its role in disease severity. **Methods and Results:** In vitro experiments on human endothelial cells (HUVEC) infected with SARS-CoV-2 showed inflammasome activation through NLRP3 and ASC puncta observation. Similarly, in vivo data from SARS-CoV-2-infected K18hAce-2 mice indicated NLRP3 and ASC activation in pulmonary endothelial cells. Additionally, murine coronaviruses MHV-3 and MHV-A59 induced inflammasome activation in endothelial cells of infected C57bl/6 mice, with no activation in *Nlrp3*^{-/-} mice, as expected. Exploring the pathways leading to NLRP3 activation, we observed reduced inflammasome activation in endothelial cells from mice lacking TNF and NOS2 signaling. Finally, we treated mice with clodronate liposomes to deplete alveolar macrophages and found that the recruitment of neutrophils and monocytes would still occur in treated infected mice. All in vivo experiments were performed following ethics approved protocol 033/2020. **Conclusions:** This study demonstrates that the betacoronaviruses activate the NLRP3 inflammasome in pulmonary endothelial cells, participating in the recruitment of neutrophils and monocytes, and potentially contributing to worse disease prognosis. NLRP3 inflammasome activation in endothelial cells appears to be a significant factor in coronavirus-induced pathology, highlighting potential targets for therapeutic intervention in severe COVID-19. **Financial support:** FAPESP, CRID, CNPq and CAPES.

Keywords: Endothelial cells;NLRP3;Coronavirus.

3137 - Category: INNATE IMMUNITY (IN)

Placental TLR10 expression in Zika virus-infected mothers: exploring its role in Congenital Zika Syndrome susceptibility

SOUSA, D.P.¹; PEREIRA, N.V.²; RAMOS, Y.Á.L.²; DE VASCONCELOS, Z.F.M.³; SATO, M.N.². 1. INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. FACULTY OF MEDICINE, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. OSWALDO CRUZ FOUNDATION, RIO DE JANEIRO - RJ - BRASIL.

Toll-like receptor 10 (TLR10) is a member of the TLR family whose function remains poorly understood, but emerging evidence suggests a predominantly anti-inflammatory role. Although still poorly characterized, TLR10 has been implicated in antiviral responses in a few studies involving different viruses, suggesting a possible role in host defense against viral infections. Zika virus (ZIKV) infection during pregnancy has been associated with severe fetal outcomes, collectively referred to as Congenital Zika Syndrome (CZS), which includes microcephaly and other developmental abnormalities. The maternal-fetal interface plays a crucial role in modulating immune responses to protect the fetus while controlling pathogens such as ZIKV. This study aims to investigate the anti-inflammatory role of TLR10 at the maternal-fetal interface during ZIKV infection. We hypothesize that TLR10 modulates the placental immune response to ZIKV. Our results demonstrate that ZIKV is capable of infecting BeWo cells, a trophoblast-derived cell line. Immunohistochemical analysis of placental tissues from ZIKV-infected mothers revealed increased TLR10 protein expression in the decidua compartment of those whose infants did not develop CZS. In contrast, the decidua from ZIKV-infected mothers whose infants did develop CZS did not show significant differences in TLR10 expression compared to basal levels. These findings suggest a potential role for TLR10 in regulating placental responses to ZIKV infection. The use of placental tissues in this study was approved by the institutional ethics committee under IRB/CAAE: 52675616.0.000.5269. Further investigations will include TLR10 neutralization using specific antibodies in BeWo cells and placental explant cultures exposed to ZIKV, as well as gene expression interference strategies to better understand TLR10's role in placental immunity.

Keywords: CD290;Toll-like receptors;Trophoblasts.

3157 - Category: INNATE IMMUNITY (IN)

Leishmania proteases are required for non-canonical cleavage of Gasdermin-D in Macrophages

CARROZZA, N.C.; FERNANDES, J.C.R.; MADEIRA, J.C.C.; ZAMBONI, D.S.. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - USP, RIBEIRÃO PRETO - SP - BRASIL.

Leishmaniasis is a disease caused by the intracellular protozoan parasite *Leishmania*, which affects humans with clinical forms ranging from localized cutaneous lesions to life-threatening visceral disease. Once inside the host, *Leishmania* resides in macrophages and manipulates their signaling pathways to avoid immune clearance. One such immune evasion strategy involves the interference with inflammatory cell death. During early infection, *Leishmania* induces the formation of a non-canonical fragment of gasdermin-D (GSDMD), which does not lead to pyroptotic cell death, suggesting an active mechanism to suppress inflammasome-mediated responses. We hypothesize that this atypical cleavage event is mediated by a parasite-derived protease capable of directly targeting host GSDMD and preventing its canonical activation. To explore this hypothesis, we infected bone marrow-derived macrophages (BMDMs) from C57BL/6 and *Gsdmd*^{-/-} mice with wild-type, protease-deficient, or genetically complemented strains of *Leishmania* major Seidman strain. The alternative GSDMD fragment was consistently detected in macrophages infected with wild-type and complemented parasites, but was absent in those infected with protease-deficient *Leishmania*, indicating a critical role for a parasite protease in this process. We are currently expanding our investigation to *Leishmania* amazonensis, where we used CRISPR-Cas9 to delete a cluster of genes encoding predicted proteases. These findings suggest that *Leishmania* employs a proteolytic strategy to reshape host cell death signaling. Ongoing efforts aim to identify the specific protease(s) involved and to determine how this contributes to parasite persistence and pathogenesis.

Keywords: Gasdermin D;Inflamassome;Parasitic protease .

3173 - Category: INNATE IMMUNITY (IN)

Scorpion Venom-Induced NETosis as a Novel Immunomodulatory Pathway during Envenomation

DE AGUIAR, A.M.¹; DOS SANTOS, G.M.²; OLIVEIRA, I.³; DA SILVA, T.A.³; PUCCA, M.B.². 1. UNIVERSIDADE DO ESTADO DO AMAPÁ, MANAUS - AM - BRASIL; 2. UNIVERSIDADE ESTADUAL PAULISTA EM ARARAQUARA, ARARAQUARA - SP - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Animal venoms are complex cocktails of bioactive molecules with potent immunomodulatory properties, profoundly influencing neutrophils, the first immune cells recruited to sites of envenomation. Upon activation, neutrophils can undergo phagocytosis, release cytokines, and form neutrophil extracellular traps (NETs), a process known as NETosis, in which DNA and granular proteins are expelled to immobilize and neutralize toxins or pathogens. This study pioneers the investigation of the ability of scorpion venoms to induce NET formation. Venoms were collected from scorpion species inhabiting the Roraima region (*Tityus silvestris*, *Brotheas ssp.*, *Rhopalurus laticauda* and a new species from Chactidae family), as well as from the most medically relevant species in Brazil, *Tityus serrulatus*. Venoms were extracted via electrical stimulation of the telson (12–20 V) under standardized conditions. Scorpion venoms from *Brotheas ssp.*, *R. laticauda*, *T. serrulatus*, and *T. silvestris* induced NETosis in all tested concentrations (10, 30 and 100 mg/mL) and in a dose-dependent way. Interestingly, the new species elicited robust NET release at low concentrations, suggesting a bell-shaped dose-response potentially linked to distinct toxin composition or receptor saturation. Notably, *T. serrulatus* venom induced approximately twice as many NETs compared to the other species, correlating with its clinical severity and toxic potency. The observed NET formation suggests that components present in these venoms can directly activate neutrophils and trigger chromatin release. These findings suggest that NETosis constitutes a significant innate immune response during scorpion envenomation, potentially contributing to both local tissue damage and systemic inflammation. Identification of the specific venom molecules responsible for neutrophil activation is critical for understanding the immunopathology of scorpionism and developing targeted therapies.

Keywords: NETosis; Scorpion Venom; Immunomodulatory Toxins.

3179 - Category: INNATE IMMUNITY (IN)

The role of lipid droplets in the innate antibacterial response of murine macrophages

SAVI, R.V.¹; PEREIRA-DUTRA, F.²; BOZZA, P.T.². 1. UFRJ - UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ - IOC, RIO DE JANEIRO - RJ - BRASIL.

Recent studies indicate that the reprogramming of lipid metabolism is a central factor during inflammatory processes. Lipid droplets (LDs) are dynamic lipid-enriched organelles that store and provide lipid substrates for diverse molecular functions. Furthermore, these organelles have a central role as platforms involved in the pathogen-host relationship. Although several studies associated usurpation of LDs by pathogenic intracellular bacterias, our data suggests that the role of LDs in bacterial infections has a pro-host molecular potential, actively participating in the antibacterial innate response. The objective of this work was to evaluate the role of LD's in the immunometabolic reprogramming and antimicrobial activity of murine macrophages. *E. coli* infection was carried out in vitro in macrophages derived from mouse bone marrow (CEUA-IOC-Fiocruz L005/20). The results show that the change in lipid metabolism induced by *E. coli* infection is associated with the pro-inflammatory profile of macrophages, a change that occurs towards the accumulation of LDs in these cells. This phenomenon was observed both in *E. coli* infected or LPS+INF γ -stimulated macrophages. Furthermore, our data reveal that modulation of LDs biogenesis affects the killing capacity of murine macrophages against *E. coli* infection. Moreover, inhibition of LDs accumulation impacts the proinflammatory and antibacterial response by inhibiting the synthesis of prostaglandin E₂ (PGE₂), interferon- β (IFN β) and nitric oxide (NO) and decreasing the expression of interferon stimulated antimicrobial proteins cathelicidin (Camp), viperin and IRGM-3. Taken together, our data demonstrated that LDs play an important role in the innate antibacterial response of macrophages through their lipid mediator production machinery, as well as through the compartmentalization of antimicrobial proteins.

Keywords: lipid droplet; macrophage; bacterial infection.

3185 - Category: INNATE IMMUNITY (IN)

AIM2 INFLAMMASOME ACTIVATION CONTRIBUTES TO THE PATHOGENESIS OF BETACORONAVIRUS INFECTION

PEREIRA, K.A.S.¹; RODRIGUES, T.S.²; LOPES, F.T.¹; HERRERA, A.J.R.³; COSTA, V.V.⁴; FABRO, A.T.³; ZAMBONI, D.S.¹. 1. DEPARTMENT OF CELLULAR AND MOLECULAR BIOLOGY. RIBEIRÃO PRETO SCHOOL OF MEDICINE, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO (FMRP/USP), RIBEIRÃO PRETO - SP - BRASIL; 2. DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY, FEDERAL UNIVERSITY OF SANTA CATARINA (UFSC), BRAZIL, FLORIANOPOLIS - SC - BRASIL; 3. DEPARTMENT OF PATHOLOGY AND FORENSIC MEDICINE. RIBEIRÃO PRETO SCHOOL OF MEDICINE, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO (FMRP/USP), RIBEIRÃO PRETO - SP - BRASIL; 4. DEPARTMENT MORPHOLOGY. FEDERAL UNIVERSITY OF MINAS GERAIS (UFMG), BRASIL, BELO HORIZONTE - MG - BRASIL.

Introduction: Severe forms of COVID-19 have been associated with an exacerbated and dysregulated immune response, with the release of inflammatory cytokines. Evidence suggests activation of the AIM2 inflammasome during SARS-CoV-2 infection. However, the mechanisms responsible for this activation, the cell types involved, and the pathophysiological relevance of this process are still not fully understood. **Objective:** To evaluate the AIM2 inflammasome activation during betacoronavirus infection and its role in disease progression. **Methods and Results:** Initially, K18-hACE2 transgenic mice were infected with SARS-CoV-2 (10⁴ PFU, intranasally) and evaluated AIM2 activation in their lungs at 3 dpi (CEUA nº 1346/2024). AIM2 activation was evidenced by puncta formation in lung tissue sections, detected by IF. Additionally, *in vitro* assays using human bronchial epithelial cells (Calu-3) infected with SARS-CoV-2 (MOIs 0.1 and 1) also demonstrated activation of AIM2, as evidenced by puncta formation at 8 hpi. To further investigate the role of AIM2 in disease progression, wild type (WT) or AIM-2 deficient (*Aim2*^{-/-}) mice were intranasally infected with the betacoronavirus MHV-A59 (10⁴ PFU), a murine model for COVID-19, to assess survival, body weight, and temperature variation. Interestingly, *Aim2*^{-/-} mice showed lower mortality rate after MHV-A59 infection compared to WT C57BL/6 mice. Although no significant differences in weight loss were observed, WT mice showed a more pronounced drop in body temperature on 8 dpi. In vivo infections in K18-hACE2 *Aim2*^{-/-} mice with SARS-CoV-2 are ongoing. **Conclusion:** These preliminary findings indicate that betacoronavirus infection triggers AIM2 activation in lung epithelial cells, and suggest that AIM2 deficiency may confer protection against MHV-A59-induced disease in a murine model. Based on these results, we will investigate AIM2 activation in response to SARS-CoV-2, focusing on identifying the epithelial cell subtypes involved.

Keywords: Epithelial cells; AIM2, Inflammasome, ; Betacoronavirus.

3224 - Category: INNATE IMMUNITY (IN)

NON-CANONICAL AUTOPHAGY IS REQUIRED FOR STING ACTIVATION IN RESPONSE TO PHAGOSOMAL BACTERIA

TERREL, D.L.A.; CUNHA, L.D.. UNIVERSIDADE DE SAO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Introduction: Non-canonical autophagy pathways can be engaged to promote host antimicrobial responses. In macrophages, for instance, LC3-associated phagocytosis (LAP) pathway employs components such as Rubicon (RUBCN), NOX2, and ATG proteins (including ATG5 and ATG16L1) to conjugate autophagy effector LC3 onto phagosome membrane. LAP targets several pathogens, including bacteria, and shapes effector functions, from cargo degradation to the modulation of gene expression. Another common response to internalized pathogens is STING-dependent type I interferon (IFN) production, triggered by recognition of cytosolic DNA or bacterial cyclic dinucleotides. However, the mechanisms allowing sensing of these agonists from phagosome-sequestered pathogens remain elusive. Here, we explore if LAP regulates STING activation in response to bacteria, using *Listeria monocytogenes* (*Lm*) and *L. innocua* (*Li*) as models. **Methods:** Wild-type, *Rubcn*^{-/-} or *Atg16l1*^{ΔWD40} (a mutant lacking the C-terminal domain of ATG16L1 required for binding to single-membranes during non-canonical autophagy only) bone marrow-derived macrophages (BMDM) infected by *Lm* or *Li*. **Results:** *Ifnb1* expression and IFN-β secretion in response to wild-type *Lm*, which rapidly escapes phagosomes, was not affected in *Rubcn*^{-/-} or *Atg16l1*^{ΔWD40} BMDM. STAT1 phosphorylation (p-STAT1) in response to IFN-β was similar between *Rubcn*^{-/-}, *Atg16l1*^{ΔWD40} and control BMDM. In agreement, no difference in the levels of p-STING and p-TBK1, and a mild reduction in p-IRF3 were found in *Rubcn*^{-/-} or *Atg16l1*^{ΔWD40} BMDM. However, disruption of ATG16L1 binding to phagosome, but not depletion of RUBCN, impaired STING-TBK1-IRF3 phosphorylation, IFN-β production and STAT1 activation in response to *Lm* mutants that fail to escape the phagosome (*Δhly*, and *ΔhlyΔplcAΔplcB*) or non-pathogenic (*Li*) strains. **Conclusion:** Our results suggest that a non-canonical autophagy pathway, distinct from LAP, regulates STING activation upon sensing of phagosomal pathogens.

Keywords: Non-canonical autophagy; STING; *Listeria monocytogenes*.

3228 - Category: INNATE IMMUNITY (IN)

Inflammasome activation contributes to the immunopathogenesis of Erythema Nodosum Leprosum (ENL)

ROSA, T.L.S.A.¹; CALVO, T.L.²; DIAS, A.A.¹; PISCULICH, D.C.B.¹; DE MACEDO, C.S.¹; MOREIRA, M.D.B.P.¹; SARNO, E.N.¹; PEREIRA, V.S.¹; PESSOLANI, M.C.V.¹. 1. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO D'OR DE PESQUISA E ENSINO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Leprosy, caused mainly by *Mycobacterium leprae* (ML), can be interrupted in 30–50% of multibacillary patients by acute inflammatory episodes known as Erythema Nodosum Leprosum (ENL), which worsen peripheral nerve damage. Although neutrophilic infiltration in new skin lesions is a hallmark of ENL, the mechanisms triggering these episodes remain unclear. Evidence suggests a key role of innate immunity, marked by elevated sera levels of IL-1 β in ENL patients. IL-1 β is processed by the inflammasome complex, which involves cytosolic sensors like NLRP3 and effectors such as caspase-1 and gasdermin D (GSDMD). Thus, this work investigates the involvement of the inflammasome in ENL immunopathogenesis. **METHODS:** We performed RNA-seq on whole blood from multibacillary patients without reaction (NR), with ENL before and after 7 days of thalidomide treatment (Thal). Circulating IL-1 β , IL-18, and GSDMD levels were measured by ELISA. Inflammasome gene expression was assessed in skin and blood samples via RT-qPCR and IL-1 β and caspase-1 cleavage via western blotting. Skin biopsies from patients with ENL $\geq$ 2 years post-MDT (ENL2y) were also analyzed for ML DNA (qPCR) and mycolates (LC-MS). Finally, PBMCs from healthy donors were stimulated with ML PAMPs to assess inflammasome activation in vitro. **RESULTS:** ENL patients showed enriched inflammasome signatures in RNA-seq and increased *IL1B*, *CASP1*, *CASP4*, *AIM2*, and *NLRP3* expression. Circulating IL-1 β and GSDMD levels were elevated in ENL, and inflammasome activation was confirmed in skin lesions, decreasing after thalidomide. ML DNA and mycolates persisted in ENL2y lesions, which also showed inflammasome activation. ML PAMPs triggered IL-1 β release by PBMCs. **CONCLUSION:** Our findings demonstrate inflammasome activation in ENL both systemically and in skin lesions, likely driven by *M. leprae* PAMPs. Thalidomide's efficacy may relate to its modulation of this pathway, highlighting new potential therapeutic targets for ENL.

Keywords: Inflammasome; Leprosy; Erythema Nodosum Leprosum.

3240 - Category: INNATE IMMUNITY (IN)

Characterization of genetic markers related to the pathogenesis of COVID-19: role of type I IFN receptors, IFNAR1 and IFNAR2, in the disease outcomes and associated comorbidities.

DOS SANTOS, C.D.O.; DE SÁ, N.B.R.; DE LUCA, D.M.; DO NASCIMENTO, R.L.L.D.; GOULART, M.N.G.; ALVES, M.R.; GERALDO, K.M.; RIBEIRO, M.P.D.; CARDOSO, S.W.; GRINSZTEJN, B.; VELOSO, V.G.; CAZOTE, A.D.S.; ALMEIDA, D.V.; GRIPP, C.B.W.G.; CÔRTEZ, F.H.; MORGADO, M.G.. IOC - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Background: Several SNPs have been associated with COVID-19 outcomes, particularly those linked to common comorbidities such as hypertension (SAH), diabetes (DM), obesity, and COPD. This study evaluated four SNPs in the IFNAR1 and IFNAR2 genes, involved in the SARS-CoV-2 signaling pathway, to explore their association with COVID-19 outcomes and identify potential biomarkers of disease pathogenesis.

Methods: 384 inpatients were followed at the Hospital Center at INI/FIOCRUZ, RJ, Brazil. IFNAR1 (rs1012335:rs1012334) and IFNAR2 (rs2834158:rs2236757) genotyping SNPs were determined by PCR. Risk factors for progression to death and/or mechanical ventilation (MV) use were analyzed using Cox's proportional hazard models. Associations were estimated by unconditional logistic regression models.

Results: Related to clinical progression, the IFNAR2 CA haplotype was associated with a slow risk of progression to death (aHR=0.098; P=0.032). Carrying the IFNAR1 CA haplotype was associated with a high risk of progression to the use of MV (aHR=19.315 (4.139-90.131); P=0), as a proxy of disease severity. However, no significant associations were observed for progression to discharge or death with the genotyped SNPs; however, relevant associations were found for COVID-19-related comorbidities. Regarding risk for COPD, associations were observed for the IFNAR1 rs1012335 C/C genotype (aOR=6.36; P=0.0138), the C allele (aOR = 1.06; P=0.004), and the C-carrier (aOR=4.45; P=0.0223). Related to obesity, in the IFNAR2 rs2834158 the C/T genotype (aOR=0; P=0.0343) and T-carrier (aOR=0.55; P=0.048) were associated with protection. Moreover, in the IFNAR1 rs1012335, G-carrier (aOR=0.52; P=0.0477) was associated with protection against DM, while in the IFNAR1, the GT haplotype (aOR=1.84; P=0.014) was associated with increased risk for SAH.

Conclusion: This study contributes to the understanding of the influence of IFNAR1/2 SNPs on the clinical course of COVID-19 and its relevant comorbidities.

Keywords: COVID-19; Single-nucleotide polymorphisms (SNP); Comorbidities.

3247 - Category: INNATE IMMUNITY (IN)

Long COVID patients with cognitive impairment show platelet hyperactivation and hyperresponsiveness

DOS SANTOS, J.P.R.S.¹; SOUZA, L.R.²; DE PAULA, M.M.L.¹; OLIVEIRA, R.T.R.¹; SHIGAEFF, N.¹; TOSCANO, E.C.D.B.¹; WEYRICH, A.³; BOZZA, P.T.²; HOTTZ, E.D.¹. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. OKLAHOMA MEDICAL RESEARCH FOUNDATION, OKLAHOMA CITY - ESTADOS UNIDOS DA AMERICA.

Long COVID involves persistent symptoms after the acute phase of COVID-19. Previous studies from our group have shown platelet hyperactivation in severe cases, but there was a lack of evidence in patients with cognitive impairment after a mild acute illness. Our hypothesis is that platelet hyperactivation and hyperresponsiveness persist in long COVID. We conducted a prospective study (CEP:47489021.2.0000.5147), evaluating platelets at 198 (IQR 98.86-382.9), 376 (IQR 313.3-512) and 472 (IQR 421.8-568.8) days post-acute infection in a cohort of long of patients classified as long COVID with cognitive impairment after evaluation by trained neuropsychologists. Two control groups were used in this study, the first consisting of pre-pandemic individuals who had no contact with SARS-CoV-2 and the second consisting of individuals who recovered from the acute phase without developing cognitive impairment. To investigate platelet activation, isolated platelets were labeled for the platelet activation markers CD62p, CD63, PAC-1 and tissue factor (TF) and analyzed by flow cytometry. In addition, to investigate platelet responsiveness, they were stimulated with thrombin and labeled with anti-CD62 and anti-CD63 monoclonal antibodies. The supernatants were analyzed by ELISA for cytokines. Our results have shown that patients with long COVID showed platelet hyperactivation, with higher expression of CD62p and CD63 compared to pre-pandemic. Platelets also showed hyperresponsiveness to thrombin (higher expression of CD62p and CD63 vs. pre-pandemic). The expression of CD62p decreased over time, but remained higher than the control. When observing cytokine secretion, we see a reduction in PF4 levels compared to the recovered group. Thus, patients with long COVID and cognitive impairment after mild COVID-19 have persistent platelet hyperactivation and hyperresponsiveness. But, the reduction in CD62p suggests gradual recovery, but a longer follow-up period is needed for confirmation.

Keywords: Platelet hyperactivation;Platelet hyperresponsiveness;Long COVID.

3252 - Category: INNATE IMMUNITY (IN)

Exploring the Action of FAHFA Family Members in Modulating Macrophage Function and Polarization: An Immunometabolic Approach

TEIXEIRA, M.L.D.C.M.¹; CASTELUCCI, B.G.¹; MACÊDO, P.E.F.²; BERÇOT, M.R.¹; PILLI, R.A.¹; VIEIRA, P.M.M.D.M.¹. 1. UNIVERSIDADE ESTADUAL DE CAMPINAS (UNICAMP), CAMPINAS - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Fatty acid esters of hydroxyl fatty acids (FAHFAs) are a class of bioactive lipids with anti-inflammatory and antidiabetic properties. In this study, we investigated the role of a specific FAHFA family member, here called FAHFA1, which is enriched in metabolic tissues, in modulating the inflammatory and metabolic function of bone marrow-derived macrophages (BMDMs). Upon LPS stimulation, FAHFA1 reduced TNF- α secretion by macrophages. To elucidate the pathways involved in this effect, we used specific inhibitors targeting key metabolic routes. The loss of the anti-inflammatory effect of FAHFA1 upon inhibition of β -oxidation (etomoxir), calcium homeostasis (dantrolene), glutaminolysis (DON), and mitochondrial metabolism (UK5099, oligomycin) suggests that its action is strongly dependent on oxidative metabolism and mitochondrial integrity. In contrast, inhibition of glycolysis and the pentose phosphate pathway did not impair FAHFA1 activity. Ongoing experiments using different concentrations of FAHFA1 (2 μ M and 20 μ M) aim to determine the optimal dose-response and its physiological relevance. These findings indicate that FAHFA1 promotes metabolic reprogramming of macrophages toward an anti-inflammatory profile in a mitochondria-dependent manner, highlighting its potential as an immunometabolic modulator in contexts such as obesity and insulin resistance.

Keywords: Macrophage;FAHFAs;Inflammation.

3253 - Category: INNATE IMMUNITY (IN)

MECHANISMS OF MITOCHONDRIAL FRAGMENTATION AND SIGNALING INDUCED BY HEME IN MACROPHAGES

PRESTES, E.B.; ALVES, L.S.; VIDAL, V.M.; DA SILVA, E.R.; GAMA, A.M.D.S.; BOZZA, M.T.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Introduction. Heme, a hemoglobin cofactor, is released during hemolysis and triggers reactive oxygen species (ROS) production, including mitochondrial ROS (mtROS), exerting pro-inflammatory effects on macrophages. Early activation of spleen tyrosine kinase (Syk) and MAPKs (ERK, JNK, p38) upon heme exposure is ROS-dependent and essential for cytokine release. Heme also disrupts mitochondrial respiration and induces mitochondrial fission within minutes, indicating a potential role for mitochondria in signaling. This study investigates the mechanisms of heme-induced mitochondrial fragmentation and its contribution to Syk and MAPK activation.

Methods and Results. In BMDMs, MAPK activation occurs in the cytosol, whereas Syk is phosphorylated in a mitochondria-enriched fraction. Syk partially contributes to mitochondrial fragmentation, but severe mitochondrial alterations induced by heme - such as disruption of the inner mitochondrial membrane observed by electron microscopy - suggest that additional mechanisms are involved. Mitochondrial fission is a typical response to acute stress, namely ROS production and membrane depolarization, both observed in heme-stimulated BMDMs. However, neither ROS scavenging nor blocking the mitochondrial permeability transition pore (mPTP) prevented fragmentation. Chelating intracellular Ca^{2+} even worsened it. Notably, only inhibition of complex III of the electron transport chain (ETC) preserved the fused mitochondrial network after heme exposure, despite increased ROS levels. Inhibition of ETC complexes also impaired MAPK, but not Syk, activation, suggesting their key role in heme-induced signaling.

Conclusion. Heme rapidly disrupts mitochondrial function and structure, initiating a complex signaling response in macrophages. Collectively, our results suggest that mitochondrial fragmentation may represent an early adaptive response to heme-induced stress, limiting damage and promoting mitophagy.

Financial support: CAPES and FAPERJ.

Keywords: Heme;Mitochondria;Fragmentation.

3260 - Category: INNATE IMMUNITY (IN)

TLR7 AND TLR8 GENETIC VARIANTS ARE LINKED TO COMORBIDITIES AND INCREASED RISK OF SEVERE COVID-19 AND DEATH

DE LUCA, D.M.; DE SÁ, N.B.R.; DOS SANTOS, C.D.O.; DO NASCIMENTO, R.L.L.D.; GOULART, M.N.G.; ALVES, M.R.; GERALDO, K.M.; RIBEIRO, M.P.D.; CARDOSO, S.W.; GRINSZTEJN, B.; VELOSO, V.G.; CAZOTE, A.D.S.; ALMEIDA, D.V.; GRIPP, C.B.W.G.; CÔRTEZ, F.H.; MORGADO, M.G.. INSTITUTO OSWALDO CRUZ - FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Background: Single-nucleotide polymorphisms (SNPs) in immune-related genes influence COVID-19 severity. Toll-like receptors (TLRs), particularly TLR7 and TLR8, play a key role in recognizing viral particles and activating the innate immune response. Here, we investigated four SNPs in the TLR7 and TLR8 genes in SARS-CoV-2-infected patients to identify potential biomarkers of disease progression.

Methods: The study included 384 inpatients of the Hospital Center for COVID-19 (INI/FIOCRUZ). SNPs were genotyped by RT-PCR. Associations with COVID-19 death/discharge were analyzed using time-to-event analyses to mechanical ventilation (MVS), death, or MVS+death by Cox proportional hazard models, and associations with comorbidities were assessed using unconditional logistic regression.

Results: SNP analysis in relation to COVID-19-related comorbidities showed that carrier-T in TLR7 rs179008 (aOR=0.4; P=0.035) was associated with a reduced risk of diabetes mellitus in women. For men, carrier-G in TLR7 rs179009 (aOR=0.36; P=0.029) was linked to a decreased risk of systemic arterial hypertension. No significant associations were identified between TLR8 SNPs and comorbidities. In the time-to-event analysis, carrier-T (aHR=0.474; P=0.037) in TLR7 rs179008 was associated with a reduced risk of death in both genders. In contrast, C/G genotype in TLR8 rs3764879 was linked with faster progression to MVS (aHR=2.09; P=0.045), death (aHR=6.84; P<0.001) and MVS+death (aHR=3.21; P=0.016). Similarly, A/G genotype in TLR8 rs3764880 was also associated with faster progression to MVS (aHR=2.09; P=0.045), death (aHR=6.84; P<0.001) and MVS+death (aHR=3.21; P=0.016). Carrier-G in both TLR8 SNPs was associated with a higher risk of death (aHR=6.32; P<0.001) and MVS+death (aHR=3.19; P=0.014).

Conclusions: Polymorphisms in the TLR7 gene are associated with relevant comorbidities related to COVID-19. Moreover, SNPs in TLR8 genes are linked to progression to critical disease and death.

Keywords: COVID-19;Single-nucleotide polymorphisms (SNPs);Risk factors.

3270 - Category: INNATE IMMUNITY (IN)

Adipocyte ATGL deletion alters fat distribution but fails to prevent sarcopenic in aged mice

FERREIRA, F.B.; SEGURA, G.G.; DE OLIVEIRA, F.A.; CASSIANO, A.C.M.; LEIRIA, L.O.S.. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO FMRP/USP, RIBEIRÃO PRETO - SP - BRASIL.

Aging leads to changes in body composition, such as reduced lean mass and fat redistribution, favoring the development of sarcopenic. When associated with obesity, this condition is referred to as sarcopenic obesity. Dysfunctional adipocytes, common in aging, sedentary behavior, and obesity, exhibit altered lipolysis and reduced fatty acid uptake, promoting ectopic fat accumulation in skeletal muscle. Lipid infiltration contributes to sarcopenic. Although ATGL deletion has shown beneficial effects in obesity, its impact on aged muscle remains poorly understood. The aim of this study is to investigate the impact of ATGL enzyme deletion in adipocytes on body composition and muscle strength in young and aged animals. We used control mice (ATGL^{Flox/Flox}) and mice with specific deletion of ATGL in mature adipocytes (Adipoq^{Cre}/ATGL^{Flox}), and evaluated body composition and physical performance at 10 and 24 months of age under a chow diet. Although no differences in total body weight were observed between young and aged animals in the control group, significant changes in body composition were detected with aging. Aged animals showed reduced lean mass and increased body fluid, with no absolute change in fat mass, but a shift toward greater visceral lipid deposition. The reduction in lean mass was confirmed by decreased quadriceps and gastrocnemius weights and was reflected in lower grip strength, incremental load test performance, and maximal strength. In ATGL-KO animals, fat mass remained elevated from 10 months of age, with aging leading to changes in fat distribution and reduced visceral fat. These animals had lower lean mass since youth, without significant decline with aging. However, they still experienced age-related strength loss. Both groups developed sarcopenic by 24 months of age, suggesting that ATGL deletion in adipocytes does not prevent age-associated sarcopenic.

Keywords: : lipolysis;sarcopenic;aging.

3271 - Category: INNATE IMMUNITY (IN)

Investigation of the role of non-canonical autophagy in IL-4R α signaling in response to efferocytosisDOS SANTOS, M.D.R.Z.¹; TERREL, D.L.A.¹; ROCHA, M.F.²; MOREIRA, I.Z.¹; CUNHA, L.D.¹. 1. UNIVERSIDADE DE SÃO PAULO - USP, RIBEIRÃO PRETO - SP - BRASIL; 2. GHENT UNIVERSITY, GHENT - BELGICA.

Control of tissue damage and restoration of physiological conditions in response to infectious agents or sterile insults is a complex process in which macrophages play a fundamental role by secreting mediators with anti-inflammatory and pro-tissue repair effects. The efficient clearance of dead cells, a process known as efferocytosis, is crucial for resolution and tissue homeostasis, and defects in efferocytosis are reportedly pathogenic. Efferocytosis by macrophages can prime signaling triggered by Th2 cytokines IL-4 and IL-13 through the IL-4 receptor alpha (IL-4R α), programming these cells for tissue repair functions. However, the mechanisms responsible for integrating these signals remain unknown. The efficient degradation of engulfed dead cells by macrophages requires a non-canonical autophagy process called LC3-associated phagocytosis (LAP), in which ATG8 family proteins, such as LC3, are conjugated to the membrane of the phagosome. Here, we aim to investigate the mechanisms associated with the pro-resolutive function of macrophages in the context of efferocytosis. Our hypothesis is that LAP promotes IL4R α signaling in response to efferocytosis. Corroborating our hypothesis, our preliminary data indicates a reduction on the transcription of *Il4ra* and surface expression of IL4-R α in bone marrow-derived macrophages (BMDM) deficient in Rubicon (RUBCN), a component of non-canonical autophagy pathway, in response to stimulation with apoptotic cells. To gain insight *in vivo*, we evaluated the role of RUBCN using a conditional deletion model and induction of peritonitis with thioglycolate. We observed reduced IL4-R α expression on the surface of peritoneal macrophages from LysM-Cre⁺ *Rubcn*^{fl/fl} mice compared to WT littermate controls. Moving on, we will determine if efferocytosis is a cell autonomous mechanism regulating IL-4R α expression, and if it plays a role in resolution during parasitic infection *in vivo*.

Keywords: IL-4 receptor alpha (IL-4R α);efferocytosis;non-canonical autophagy.

3278 - Category: INNATE IMMUNITY (IN)

Neutrophil Extracellular Traps are important for the degradation of amyloid fibrils in vivo: implications for amyloidosis

MARTINS, A.D.S.¹; PIRES, T.R.C.¹; GUIMARÃES, F.V.²; MATTOS, T.R.F.³; SARAIVA, E.¹; MARTINS, P.M.R.E.S.²; FOGUEL, D.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Amyloid fibrils (AF) are composed of misfolded proteins organized in a β -cross structure and are often associated with neurodegenerative diseases. Neutrophil Extracellular Traps (NETs) are decondensed chromatin fibers decorated with antimicrobial proteins, which, while key to immune defense, are implicated in various pathologies. Our group was the first to show that, in vitro, human neutrophils incubated with AF release NETs in a manner NOX-2-dependent, and that neutrophil elastase (NE) present in NETs digests AF, forming cytotoxic oligomers. We also found that NET-markers were colocalized with amyloid deposits in lung and skin biopsies from patients with systemic amyloidosis, suggesting the potential in vivo significance of this interaction. Now, these studies were extended to animal models where AF were instilled into the lungs of wild-type (WT) and NOX-2 knockout (KO) mice. Eight hours after AF instillation, both WT and KO mice showed pronounced neutrophil infiltration, with NETs formation being observed only in WT mice. Interestingly, Congo red-positive amyloid-like deposits persisted in the lungs of KO animals but were absent in WT counterparts, suggesting that NET-associated proteases contribute to the clearance of AF from the lung. Lung function was evaluated through measurements of elastance and resistance, where we observed that elastance was compromised while AF remained in the lungs of both mice. As AF were progressively cleared in WT mice, lung function improved, whereas KO mice, in which AF persisted, continued to exhibit impaired elastance. In vitro experiments demonstrate that both supernatant of PMA-activated neutrophils containing NETs and NE are capable of digesting AF into smaller species, further reducing the binding of these structures to Thioflavin T. Collectively, these findings indicate that AF disrupt lung function and that NETs, triggered by these fibrils, facilitate their degradation and protect lung tissue from further damage. Keywords: Amyloid Diseases;Inflammation;Neutrophil Extracellular Traps.

3326 - Category: INNATE IMMUNITY (IN)

Identification of bio-active peptides associated with wound healing properties in ocellate river stingray (*Potamotrygon motoro*) mucus

MOYSES, M.N.¹; FALLA, M.V.A.¹; CASTANHOLA-DIAS, L.²; KATO, E.E.¹; DE OLIVEIRA, L.³; DA SILVA, J.O.¹; DOS SANTOS, N.G.P.¹; PIMENTA, D.C.¹; COELHO, G.R.¹. 1. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL; 2. INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA, MANAUS - AM - BRASIL; 3. SHIMADZU DO BRASIL, BARUERI - SP - BRASIL.

South American freshwater stingrays (subfamily Potamotrygoninae) are medical interest's animals widely distributed in Brazilian watersheds, which are capable of secreting mucus, rich in organic compounds, all over their body surface as a form of passive defense. Recent studies have shown that fish mucus has important biological activities for the immunity of these animals. However, little is known about the biochemical composition and biological activity of stingray mucus. In this context, this work aims to biochemically characterize the peptides of the mucus of the species *Potamotrygon motoro* and evaluate their biological activities. *P. motoro* mucus purification and biochemical characterization of peptides and proteins was carried out through reversed-phase high-performance liquid chromatography (RP-HPLC) and mass spectrometry, respectively. To assess biological function, crude mucus and selected fractions were tested in scratch wound healing assay using normal human fibroblast cell line (FN1). The peptidomic analysis enable the identification of peptides possibly originating from constitutive proteins, among them keratin, a protein also identified in the proteomic analysis, which also identified actin, histone H4 and PcfHB. The mucus showed wound healing activity by modulating fibroblast migration in a concentration-dependent manner, the same activity was observed on low mass fraction and fraction 6. This is the first report describing the composition of *P. motoro* ray mucus, highlighting its peptide/protein composition. The discovery of the new peptides indicates that fragments of constitutive proteins may be acting as cryptides and playing important biological roles in the immunity of these animals, such as tissue repair in the face of injuries.

Keywords: Mucus;Peptides;Wound healing.

3348 - Category: INNATE IMMUNITY (IN)

ROLE OF ADRENERGIC RECEPTORS ON GROUP 3 INNATE LYMPHOID CELLS

BOS, N.N.¹; CIPELLI, M.¹; SOUZA, K.B.D.S.¹; FACHI, J.²; VINOLO, M.A.R.³; BASSO, A.S.⁴; CAMARA, N.O.¹; ARAUJO, L.P.¹. 1. DEPARTAMENTO DE IMUNOLOGIA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS ICB- USP, SÃO PAULO - SP - BRASIL; 2. DEPARTMENT OF PATHOLOGY & IMMUNOLOGY, WASHINGTON UNIVERSITY IN ST. LOUIS, SAINT LOUIS - ESTADOS UNIDOS DA AMERICA; 3. DEPARTAMENTO DE BIOLOGIA, INSTITUTO DE BIOLOGIA IB-UNICAMP, SÃO PAULO - SP - BRASIL; 4. DEPARTAMENTO DE MICROBIOLOGIA, IMUNOLOGIA E PARASITOLOGIA DA EPM-UNIFESP, SÃO PAULO - SP - BRASIL.

The microbiome is the community of microorganisms associated with the human body, and it is widely studied in the gut. In this context, distinct subsets of immune cells reside in the intestine, where they play key roles in distinguishing commensals from pathogens. Group 3 innate lymphoid cells (ILC3s) and CD4⁺ Th17 cells are crucial in autoimmunity and bacterial responses. Additionally, enteric nervous system innervation is essential for gut-brain axis homeostasis. To achieve this, a proper immune response—possibly modulated by neural inputs—is critical for host health. In this scenario, the expression of neurotransmitter and neuropeptide receptors on immune cells may allow them to sense sympathetic nervous system signals.

After careful review of the literature and public datasets, we analyzed RNA-seq data from sorted ILC3s to assess catecholaminergic receptor expression. In steady-state mice, ILC3s express alpha-2A (ADRA2A) and beta-2 (ADRB2) adrenergic receptors, suggesting potential responsiveness to noradrenaline. Based on this, we used the MNK cell line (an ILC3 in vitro model) to explore their function. These cells were cultured in the presence of IL-2 and IL-7, and upon IL-23 stimulation, they produced IL-17 and IL-22, resembling ILC3 activity. Ongoing RT-qPCR experiments are being performed to evaluate adrenergic receptors expression in MNK cells. Since they may respond to sympathetic cues, we are testing ADRA2A and ADRB2 agonists and antagonists to evaluate their roles in ILC3 activation and cytokine production. Future experiments will examine how adrenergic signaling affects ILC3s during intestinal infection.

Understanding how neuromodulation affects ILC3s is crucial, as it may influence inflammatory bowel diseases. We hypothesize that adrenergic signaling modulates IL-22 and IL-17 production in ILC3s, thereby impacting host protection during early stages of intestinal infection.

Keywords: gut-brain axis; ILC3s; infection.

3357 - Category: INNATE IMMUNITY (IN)

Copaiba Oleoresin Bioprospecting: Standardizing an In Vitro Immune Complex Deposition Model to Evaluate Neutrophil Activation and Extracellular Vesicle Release

DE SOUZA, M.¹; MACIEL, L.D.O.²; TUCCI, L.F.F.²; BORGES, A.O.³; AZEVEDO, P.N.⁴; SORGI, C.A.⁴; GASPARI, P.D.M.²; CIANCAGLINI, P.⁴; DE OLIVEIRA, E.C.P.⁵; MACHADO, C.M.M.². 1. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO DA UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO DA UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DO AMAZONAS, MANAUS - AM - BRASIL; 4. FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO DA UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 5. UNIVERSIDADE FEDERAL DO OESTE DO PARÁ, SANTARÉM - PA - BRASIL.

Chronic inflammatory diseases (CIDs) are a significant global public health concern, often associated with reduced quality of life and limited therapeutic options. In this context, medicinal plants remain an important source of bioactive compounds. Copaiba oleoresin (CpO), extracted from the Amazonian species of the genus *Copaifera* (*Copaifera* spp.), is traditionally used for its anti-inflammatory properties; however, its mechanisms of action remain poorly understood. Neutrophils play a central role in inflammation and contribute to CID pathophysiology through effector functions, which include phagocytosis, production of reactive oxygen species (ROS), degranulation, and the release of neutrophil extracellular traps (NETs) and extracellular vesicles (EVs). EVs mediate intercellular communication and participate in the regulation of inflammatory response. This study aimed to investigate the effects of CpO and its volatile fraction (VF) on neutrophil oxidative metabolism and EV secretion in an *in vitro* model of immune complex (IC) deposition, a process involved in several CIDs. Human neutrophils from healthy donors were exposed to CpO (5, 10, and 15 µg/mL) and VF (12.5, 25, 50, 100, and 150 µg/mL), based on prior viability data (MTT and LDH assays), and incubated on plates with or without immobilized ICs. ROS production was quantified by chemiluminescence, and EVs were isolated by ultracentrifugation and analyzed using nanoparticle tracking analysis (NTA). The IC model: (i) promoted neutrophil activation, as indicated by increased ROS levels, with or without CpO or VF treatment at the tested concentrations; and (ii) enhanced EV secretion. This model is currently being used to further explore the potential of CpO in modulating inflammatory responses mediated by neutrophil-derived EVs in the context of IC deposition.

Keywords: Neutrophil; Copaiba oleoresin; Extracellular vesicles.

3373 - Category: INNATE IMMUNITY (IN)

Analysis of *Euphorbia tirucalli* Latex: Anti-Inflammatory Effects and Nitric Oxide Production in RAW 264.7 Macrophages

LIMA, A.C.P.¹; FERREIRA, A.C.A.²; CORREIA, I.P.R.²; FERNANDES, A.M.S.²; MEYER, R.²; DA SILVA, M.B.². 1. CENTRO UNIVERSITÁRIO JORGE AMADO, SALVADOR - BA - BRASIL; 2. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL.

Euphorbia tirucalli, commonly known as avelós or pencil tree, is a plant belonging to the Euphorbiaceae family. Its latex is rich in secondary metabolites such as triterpenes, diterpenes, and flavonoids, compounds whose biological properties are widely described in the literature, including anti-inflammatory, antitumor, and antioxidant activities. This plant is traditionally used in folk medicine to treat inflammatory diseases, ulcers, warts, and cancer. However, the presence of the diterpene phorbol, a substance known for its toxicity, represents a limitation to its therapeutic use, as it can cause skin and mucosal irritation. It is known that activated macrophages express the enzyme inducible nitric oxide synthase (iNOS) and produce nitric oxide (NO), a key inflammatory mediator. To evaluate the immunomodulatory potential of *Euphorbia tirucalli* latex in murine macrophages of the RAW 264.7 cell line. RAW 264.7 macrophage cultures were used to assess cell viability through the MTT assay. After stimulation with lipopolysaccharide (LPS), the immunomodulatory properties of the latex were analyzed by measuring nitric oxide (NO) production and the levels of pro- and anti-inflammatory cytokines (IL-6, TNF- α , and IL-1 β). An anti-inflammatory activity of *E. tirucalli* latex was observed, with significant inhibition of nitric oxide and pro-inflammatory cytokine production compared to the control group. Data were statistically analyzed, with p-values less than 0.05 considered statistically significant. *Euphorbia tirucalli* latex demonstrated the ability to inhibit nitric oxide production and modulate the release of inflammatory cytokines, indicating a potential immunomodulatory effect.

Keywords: *Euphorbia tirucalli* ;Anti-Inflammatory;RAW 264;7.

3380 - Category: INNATE IMMUNITY (IN)

Mitochondrial fission modulates CpG-induced trained immunity in macrophages

TRIOZZI, E.; BERÇOT, M.R.; SILVA, L.F.L.; BATAGLIOLI, B.G.C.; MARTINS, A.J.E.; VIEIRA, P.M.M.D.M.. LIM - LABORATÓRIO DE IMUNOMETABOLISMO ; DGEMI, UNICAMP, CAMPINAS - SP - BRASIL.

The innate immune system serves as the first line of defense against pathogens and tissue damage, orchestrating microbial clearance, debris removal, and tissue repair. Macrophages are central to these processes, dynamically modulating immune responses in both inflammatory and reparative contexts to maintain homeostasis. Innate immune cells can develop enhanced antimicrobial and signaling capabilities following initial exposure to pathogen- or damage-associated molecular patterns (PAMPs/DAMPs), a phenomenon termed trained immunity. This adaptive-like response arises from metabolic and epigenetic reprogramming, priming cells for heightened secondary responses. Mitochondria play a crucial role in these metabolic shifts, supplying energy, redox equivalents, and immunomodulatory metabolites that shape immune function. Mitochondrial homeostasis is tightly linked to dynamic morphological changes—fission and fusion—that occur in response to cellular and environmental cues. The extent to which mitochondrial dynamics influence trained immunity remains poorly understood. Here, we hypothesize that mitochondrial fission is a critical regulator of trained immunity in bone marrow derived macrophages (BMDMs). We observed that macrophages trained with CpG (Oligodeoxynucleotide 1826) exhibit upregulated Interleukin-1 β expression and reduced phosphorylation of dynamin-related protein 1 (DRP1, Ser616), suggesting diminished fission activity upon lipopolysaccharide restimulation. These observations imply that mitochondrial dynamics, particularly fission modulation, may be essential for trained immune responses. Pharmacological and genetic approaches to inhibit mitochondrial fission or fusion further affected the trained phenotype. Together, our work aims to uncover novel mechanistic insights into how mitochondrial dynamics govern trained immunity, potentially opening new avenues for therapeutic modulation of innate immune memory.

Keywords: Immunometabolism;Mitochondrial dynamics;Trained immunity.

3384 - Category: INNATE IMMUNITY (IN)

Role of Neutrophil Extracellular Traps in the Pathogenesis and Control of Pulmonary Replication of *Legionella* spp.

GUERRA, R.O.; LEANDRO, M.D.O.; RAMOS, A.D.S.; MASCARENHAS, D.P.A.; ZAMBONI, D.S.. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Legionella spp. are Gram-negative bacteria that infect humans primarily through the inhalation of contaminated aerosols, potentially leading to Legionnaires' disease, a severe and often fatal form of pneumonia. Neutrophils play a pivotal role in controlling *Legionella pneumophila* infection through the secretion of TNF, which facilitates bacterial clearance by alveolar macrophages; the generation of reactive oxygen species (ROS); and the production of immunomodulatory cytokines such as IFN- γ and IL-12. In addition to these functions, neutrophils can form extracellular traps, known as NETs (*Neutrophil Extracellular Traps*), which consist of decondensed DNA associated with histones and granular proteins that contribute to pathogen elimination. However, comprehensive investigations into NET formation during *Legionella* spp. infection, as well as the molecular signaling pathways and regulatory dynamics involved, remain limited. Our findings demonstrate that both *L. pneumophila* and *L. longbeachae* can induce NET release *in vitro*. Notably, NET formation *in vivo* was predominantly detected in C57BL/6 mice infected with *L. longbeachae*, and this response was reduced in mice deficient in IFN- γ , TNFR, and CCR2. These results suggest that, despite belonging to the same genus, *L. pneumophila* and *L. longbeachae* elicit distinct neutrophil responses with regard to NET induction. Taken together, these data highlight the need for further investigation into the molecular mechanisms involved in NET formation during *Legionella* infection. Moreover, they raise the hypothesis that certain species may modulate or even subvert this response as an immune evasion strategy. Elucidating these mechanisms may significantly enhance our understanding of *Legionella* pathogenesis.

Keywords: *Legionella*;NET;neutrophils.

3400 - Category: INNATE IMMUNITY (IN)

Effect of IL-10 release in a murine asthma model mediated by prostaglandin D2 receptor antagonism.

TASMO, N.R.A.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Eosinophils are granulocytes classically characterized as effector cells of the symptomatology of asthma. On the other hand, important immunomodulatory and even homeostatic functions have also been attributed to eosinophils due to the secretion of cytokines, such as IL-10. Eosinophils also produce and are activated by prostaglandin D2, a lipid mediator that activates its specific receptors, named DP1 and DP2. The aim of this study was to evaluate the role of the DP2 receptor in regulating the secretion of immunomodulatory molecules in the inflammatory allergic response in mice. A model of pulmonary allergic inflammatory reaction in BALB/c mice sensitized and challenged with ovalbumin was used. To study the role of selective activation of the DP1 receptor by endogenous PGD2 produced during the allergic response, mice were treated with the DP2 receptor antagonist, Cay10471. To investigate the role of IL-10 in the effects driven by DP2 antagonism, animals were treated with Cay10471 in combination with an anti-IL-10 antibody. After the allergenic challenge, we observed the characteristic bronchoalveolar eosinophilia with increased levels of PGD2; parameters that were not modified by treatment with Cay10471. The treatment also induced increased levels of lung PGE2 and serum IL-10 during the allergic reaction. It also promoted a beneficial effect on the control of pulmonary allergic inflammation, decreasing classical parameters such as bronchoconstriction, mucus production, pulmonary fibrosis, and the secretion of mediators such as LTC4, IL-13 and TGF- β . Furthermore, we found that IL-10 seems to be a key mediator in this beneficial effect, since the animals treated with the combination of Cay10471 and anti-IL-10 did not show the improvement in allergic inflammatory parameters promoted by Cay10471. We unveiled that DP2 receptor antagonism promotes protection during pulmonary allergic inflammation, by a mechanism that seems to depend, in part, on IL-10 activity.

Keywords: prostaglandin D2;IL-10;asthma.

3401 - Category: INNATE IMMUNITY (IN)

NLRP3 Inflammasome Is Activated During Mycobacterium leprae Infection

BECERRA, A.D.M.¹; DE OLIVEIRA, I.M.²; DE PAULA, N.A.¹; HERRERA, A.J.R.¹; ROSA, P.S.³; FRADE, M.A.C.¹; ZAMBONI, D.S.¹. 1. USP, RIBEIRÃO PRETO - SP - BRASIL; 2. USP, RIBEIRÃO PRETO - SP - BRASIL; 3. INSTITUTO LAURO DE SOUZA LIMA -, BAURU - SP - BRASIL.

Mycobacterium leprae, the causative agent of leprosy, is an intracellular bacterium that primarily affects the skin and peripheral nerves. Despite advances in understanding host responses to *M. leprae*, the role of inflammasomes in leprosy remains unknown. Inflammasomes are cytosolic multiprotein complexes that activate caspase-1 and promote the secretion of IL-1 β and IL-18, playing a key role in host defense. Here, we investigated the activation and functional role of inflammasomes during *M. leprae* infection. Immunofluorescence (IF) analysis of skin biopsies from leprosy patients revealed ASC and NLRP3 puncta, indicating canonical inflammasome activation. In vivo experiments using C57BL/6 (B6), NLRP3^{-/-} and Caspase-1/11^{-/-} mice showed increased bacterial loads in NLRP3-deficient animals, suggesting a protective role for this inflammasome during infection. IF analysis of the infected footpad tissue revealed an increased number of ASC and NLRP3 puncta in B6 mice, consistent with inflammasome activation. Additionally, *in vitro* infection of bone marrow-derived macrophages from B6 and NLRP3^{-/-} mice showed a reduction in IL-1 β production in the absence of NLRP3, indicating that IL-1 β secretion is partially dependent on NLRP3 and suggesting the contribution of other inflammasome pathways. Finally, ex vivo infection of human skin organotypic cultures (hOSEC) with *M. leprae* also resulted in NLRP3 puncta formation. Together, these findings provide the first evidence of inflammasome activation in leprosy. Our data suggest that NLRP3 plays a protective role during infection and point to the involvement of additional inflammasome pathways. Further studies are needed to fully understand the contribution of inflammasome signaling in leprosy pathogenesis.

Keywords: Mycobacterium leprae; inflammasome; NLRP3.

3406 - Category: INNATE IMMUNITY (IN)

Evaluation of Cell Viability and Nitric Oxide Production in Macrophage-Like RAW 264.7 Cells Treated with Oil Extract of Azadirachta indica.

DOS SANTOS, T.C.¹; RAMBO, D.F.¹; GUEDES, M.L.¹; BATISTA, R.B.¹; LIMA, A.C.P.²; FERREIRA, A.C.A.¹; CORREIA, I.P.R.¹; ANDRADE, E.B.D.S.¹; FERNANDES, A.M.S.¹; MEYER, R.¹; DA SILVA, M.B.¹. 1. UNIVERSIDADE FEDERAL DA BAHIA-UFBA, SALVADOR - BA - BRASIL; 2. CENTRO UNIVERSITÁRIO JORGE AMADO, SALVADOR - BA - BRASIL.

Introduction: *Azadirachta indica*, commonly known as neem, is a medicinal plant rich in secondary metabolites such as flavonoids, terpenoids, and limonoids, compounds widely recognized for their anti-inflammatory, immunomodulatory, and antitumor activities. These metabolites have been investigated as potential therapeutic agents, especially in the context of diseases associated with chronic inflammation, such as cancer. **Objective:** To evaluate the effects of the oil extract of *Azadirachta indica* on cell viability and nitric oxide (NO) production in macrophage-like RAW 264.7 cells. **Methodology:** RAW 264.7 cells were cultured in DMEM medium and treated with different concentrations of the *A. indica* oil extract for 24 hours. Cell viability was assessed using the MTT assay (conversion of thiazolyl blue salt). NO production was quantified in the supernatants of LPS-stimulated cultures using the Griess method. All experiments were performed in triplicate following standardized protocols. **Results:** The results showed that the extract did not reduce cell viability at any of the concentrations tested. On the contrary, at intermediate concentrations (2.5 and 200 μ g/mL), a significant increase in cell viability was observed ($p < 0.05$). At these same concentrations, a significant inhibition of nitric oxide production was also observed. These findings indicate that the extract exhibited a concentration-dependent effect, stimulating cell proliferation and modulating the inflammatory response. **Conclusion:** The *in vitro* model allowed the observation of interactions between the extract's components and immune cells, providing evidence of the pharmacological potential of *Azadirachta indica* in modulating inflammation. The results highlight the relevance of plant-derived metabolites as promising candidates in complementary therapeutic strategies aimed at inhibiting inflammatory processes.

Keywords: Azadirachta indica; anti-inflammatory; RAW 264;7

3412 - Category: INNATE IMMUNITY (IN)

Bioprospecting of Copaiba Oleoresin on Neutrophil: Chemical and Functional Characterization

MACIEL, L.D.O.¹; DE SOUZA, M.²; TUCCI, L.F.F.¹; BORGES, A.O.³; AZEVEDO, P.N.⁴; SORGI, C.A.⁴; DE OLIVEIRA, E.C.P.⁵; MACHADO, C.M.M.¹. 1. SCHOOL OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. SCHOOL OF MEDICINE OF RIBEIRÃO PRETO, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. FEDERAL UNIVERSITY OF AMAZONAS, MANAUS - AM - BRASIL; 4. SCHOOL OF PHILOSOPHY, SCIENCES AND LETTERS OF RIBEIRÃO PRETO, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 5. FEDERAL UNIVERSITY OF WESTERN PARÁ, SANTARÉM - PA - BRASIL.

The oleoresin of *Copaifera reticulata* is widely used in traditional medicine due to its well-known anti-inflammatory and antimicrobial properties. These effects are mainly linked to its high content of sesquiterpenes and bioactive lipids present in the oleoresin, which modulate biological responses. Despite its widespread use, the effects of copaiba oleoresin on immune cells – particularly neutrophils, key players in innate immunity – remain poorly characterized. This study aimed to evaluate the effects of copaiba oleoresin (CpO) and its hydrodistillation-derived volatile fraction (VF) on neutrophil viability and reactive oxygen species (ROS) production. Physicochemical characterization of the samples was performed by gas chromatography-mass spectrometry (GC-MS), identifying β -bisabolene as the major sesquiterpene. Liquid chromatography-mass spectrometry (LC-MS) analysis revealed a predominance of free fatty acids (FA), notably arachidonic acid (FA 20:4) and eicosapentaenoic acid (FA 20:5). Neutrophils were treated with different concentrations of CpO and VF for 30 or 60 minutes. The cytotoxicity of CpO and VF was evaluated using MTT and LDH assays. Both tests indicated cell viability $\geq 80\%$ at concentrations up to 25 $\mu\text{g/mL}$ for CpO and 150 $\mu\text{g/mL}$ for VF. ROS production was quantified by chemiluminescence, with or without PMA (phorbol 12-myristate 13-acetate) stimulation. It was observed that the VF had a potential effect in reducing ROS production. This study aims to identify significant effects of copaiba oleoresin (CpO) and its fractions on the modulation of neutrophil functions, which may have potential applications in the treatment of chronic inflammatory diseases.

Keywords: oleoresin;anti-inflammatory;innate immunity.

3441 - Category: INNATE IMMUNITY (IN)

Immunomodulatory Potential of Euphorbia tirucalli Latex Observed in RAW 264.7 Murine Macrophages and HCT-116 Colorectal Cancer Cells

FERREIRA, A.C.A.¹; LIMA, A.C.P.²; CORREIA, I.P.R.¹; FERNANDES, A.M.S.¹; MEYER, R.¹; COSTA, R.S.¹; DA SILVA, M.B.¹. 1. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. CENTRO UNIVERSITÁRIO JORGE AMADO, SALVADOR - BA - BRASIL.

Colorectal cancer (CRC) is one of the most prevalent neoplasms in the world. In Brazil, it ranks second in incidence among women and third among men. Conventional therapeutic approaches have high recurrence rates, which contributes to the overload of the Unified Health System. In this context, it is essential to investigate biocomposites from plant species capable of inhibiting signaling pathways associated with inflammation and carcinogenesis, providing therapeutic alternatives with fewer side effects. *Euphorbia tirucalli* contains metabolites with documented biological activity, which has encouraged new research on its therapeutic potential in cancer treatment. The objective is to evaluate, in vitro, the anti-inflammatory and antitumor potential of *E. tirucalli* latex, focusing on its ability to inhibit tumor cell growth and modulate the macrophage response against CCR. *E. tirucalli* latex was collected, and concentrations were determined from tests on *Artemia salina* at 2.5–200 $\mu\text{g/mL}$. RAW 264.7 macrophages were cultured for anti-inflammatory parameters, as well as healthy cell controls; and HCT-116 cells, CCR cells. Viability and cytotoxicity were evaluated using the MTT assay, analyzing the dose-response. After stimulation with LPS, the anti-inflammatory activity of the latex was evaluated by quantifying nitric oxide and dosing cytokines in RAW 264.7 cells. Antitumor activity was evaluated by flow cytometry in HCT-116 cells. Statistical analysis considered results significant at $p < 0.05$. The results demonstrated that *E. tirucalli* latex exhibits cytotoxic activity against HCT-116 cells without affecting the viability of RAW 264.7 macrophages. In addition, treatment with latex reduced nitric oxide production and modulated the release of inflammatory cytokines in LPS-stimulated macrophages, indicating an anti-inflammatory effect. Therefore, the results suggest that *E. tirucalli* latex has potential as a therapeutic agent with immunomodulatory and antitumor activity.

Keywords: Colorectal cancer;Euphorbia tirucalli;Anti-inflammatory.

3464 - Category: INNATE IMMUNITY (IN)

UNRAVELING DISTINCT METABOLIC MODULATIONS INDUCED BY LEISHMANIA AMAZONENSIS AND LEISHMANIA BRAZILIENSIS IN SUSCEPTIBLE BALB/C MACROPHAGES

DE SALES, J.L.¹; LEAL, A.M.L.D.C.¹; DA SILVA, G.D.¹; DE OLIVEIRA, E.C.¹; ANDRADE, C.M.¹; GONÇALVES, Í.D.S.²; PILGER, L.M.²; DE SOUZA, N.²; VIEIRA, P.M.M.D.M.³; BRODSKYN, C.I.². 1. FIOCRUZ - BA, SALVADOR - BA - BRASIL; 2. FIOCRUZ BA, SALVADOR - BA - BRASIL; 3. UNICAMP, CAMPINAS - SP - BRASIL.

Intracellular pathogens such as *Leishmania* spp. reprogram host metabolism to ensure persistence and replication. Here, we investigated how *Leishmania amazonensis* (La) and *Leishmania braziliensis* (Lb) modulate macrophage metabolism in BALB/c mice. Bone marrow-derived macrophages (BMDMs) were obtained from BALB/c mice and differentiated in vitro for 7 days in RPMI supplemented with L929-conditioned medium.

To assess species-specific metabolic requirements, prior to infection, cells were treated for 1 hour with metabolic inhibitors—glycolysis: 2-deoxy-D-glucose (2DG) and PFK15; oxidative phosphorylation: oligomycin, antimycin A, and FCCP; lipid metabolism: C75 and etomoxir—at specific concentrations determined by cell viability assays. All inhibitors significantly reduced infection index and parasite burden in Lb-infected macrophages, whereas only mitochondrial inhibitors were effective against La, revealing distinct metabolic dependencies.

Metabolic flux analysis showed no major differences in glycolytic or respiratory rates among infected groups. However, infected macrophages exhibited increased non-glycolytic acidification, reduced ATP production, and enhanced proton leak - patterns suggestive of mitochondrial stress. Mitotracker staining confirmed reduced mitochondrial membrane potential and mass, possibly reflecting enhanced mitophagy or reduced biogenesis, which remain under investigation. Although mitochondrial ROS (mtROS) are implicated in parasite control, MitoSOX assays showed no mtROS increase with inhibitor treatment. Surprisingly, CellROX staining revealed decreased total ROS in pre-treated cells. These findings suggest a ROS-independent mechanism, possibly related to nutrient restriction or bioenergetic imbalance. Together, our results open new avenues for exploring how host metabolism can be manipulated to influence parasite survival, highlighting mitochondrial function as a promising target for host-directed interventions.

Keywords: macrophage;metabolism;leishmania.

3466 - Category: INNATE IMMUNITY (IN)

Exploring the Role of Monocytes in Inflammasome Activation Induced by *Mycobacterium leprae* Antigens: Perspectives on the Immunopathogenesis of Erythema Nodosum Leprosum.

PISCULICH, D.C.B.; PIAUY, M.H.S.; DIAS, A.A.; PEREIRA, M.P.; PESSOLANI, M.C.V.; ROSA, T.L.S.A.; MOREIRA, M.D.B.P.. FUNDAÇÃO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Leprosy, caused by *Mycobacterium leprae*, is a chronic infectious disease that can evolve into acute inflammatory episodes known as erythema nodosum leprosum (ENL), characterized by systemic symptoms, nodular lesions, and elevated proinflammatory cytokines such as TNF and IL-1 β . These features suggest inflammasome activation, although the cellular mechanisms remain unclear. Intermediate monocytes (CD14⁺CD16⁺) have been implicated in IL-1 β production during infections. This study explores the role of monocytes and their subsets in inflammasome activation during ENL, aiming to identify new therapeutic targets. **METHODS:** THP-1 monocytic cells and primary monocytes from healthy donors were stimulated with *M. leprae* PAMPs (cell wall components and mycobacterial DNA), with or without thalidomide treatment. IL-1 β and IL-18 levels were measured in supernatants by ELISA. Flow cytometry was used to assess IL-1 β expression in classical (CD14⁺CD16⁻) and intermediate (CD14⁺CD16⁺) monocytes from both healthy donors and multibacillary leprosy patients, stimulated or not with *M. leprae* sonicate. **RESULTS:** *M. leprae* PAMPs increased IL-1 β production in THP-1 cells and primary monocytes, indicating inflammasome activation. This response was reduced by caspase-1 inhibition and thalidomide. Flow cytometry revealed higher frequencies of IL-1 β ⁺ monocytes in leprosy patients versus healthy individuals. Intermediate monocytes from patients showed nearly 100% IL-1 β ⁺ cells regardless of stimulation, whereas *M. leprae* sonicate induced IL-1 β expression in classical monocytes from both groups, more prominently in leprosy patients. **CONCLUSION:** These findings suggest that *M. leprae* PAMPs activate the inflammasome in monocytes, potentially contributing to ENL-related systemic inflammation. Intermediate monocytes appear to play a central role in IL-1 β production, highlighting their relevance as targets for future therapeutic interventions.

Keywords: inflamassome;monocytes;Erythema Nodosum Leprosum.

3487 - Category: INNATE IMMUNITY (IN)

HIF-1 α -dependent PCK2 repression boosts fumarate accumulation in LPS-activated macrophages

MACÊDO, P.E.F.¹; REQUIAO, R.D.²; TOLLER-KAWAHISA, J.E.³; EVANS, V.³; CASTELUCCI, B.G.²; BERÇOT, M.R.²; MCGETTRICK, A.³; DOS REIS, L.M.²; SILVA, L.F.L.²; DA SILVA, G.R.²; PFEFER, T.³; TEIXEIRA, M.L.D.C.M.²; MIN, Y.³; O'CARROL, S.³; O'NEILL, L.³; VIEIRA, P.M.M.D.M.². 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNICAMP, CAMPINAS - SP - BRASIL; 3. TRINITY COLLEGE DUBLIN, DUBLIN - IRLANDA.

Macrophage effector functions depend on reciprocal regulatory mechanisms between metabolism and immunity. However, the biochemical pathways governing inflammatory activation remain incompletely defined. Here, we identify phosphoenolpyruvate carboxykinase 2 (PCK2) - the rate-limiting enzyme in non-hepatic gluconeogenesis - as a metabolic node distinctly regulated by pro- or anti-inflammatory stimuli in macrophages. PCK2 links the tricarboxylic acid (TCA) cycle to glycolysis/gluconeogenesis by converting oxaloacetate to phosphoenolpyruvate. Bone marrow-derived macrophages (BMDMs) predominantly express the pck2 isoform over pck1, its cytosolic paralog, and IL-4, lactate, or tumor cell-conditioned media increase pck2 expression, implicating PCK2 in resolution-associated profiles. Conversely, RNA sequencing, qPCR, proteomic, and Western blot analyses show that LPS suppresses PCK2 in a HIF-1 α -dependent manner in both mouse and human macrophages. Early pharmacological inhibition of PCK2 (3-6 h) increases ACOD1 and fumarate accumulation, while decreasing the expression of pro-inflammatory cytokines *il6*, *tnf*, *il1b*, and *il12*, as well as the release of TNF α and IL-6. At 24 h, PCK2 inhibition increases TNF α and decreases IL-10, mirroring the effects of fumarate accumulation. Accordingly, PCK2 knockdown (KD) reduces IL-10 and increases TNF α and IFN β production. These findings reveal that HIF-1 α -mediated PCK2 repression induces metabolic reprogramming that shapes macrophage inflammatory responses.

Keywords: Macrophage;PCK2;HIF-1 α .

3492 - Category: INNATE IMMUNITY (IN)

Investigation of HIF-1 α activation triggered by efferocytosis in myeloid cells deficient in LC3-Associated Phagocytosis

MARTIM, K.L.G.; DOS SANTOS, D.; MOREIRA, I.Z.; CUNHA, L.D.. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

The clearance of dead cells (efferocytosis) is a primary function of the immune system, and LC3-associated phagocytosis (LAP) is a non-canonical autophagy pathway that contributes to this process. LAP requires the recruitment of some, but not all, components of macroautophagy. One of LAP's exclusive components is Rubicon (RUBCN), a protein that binds inner plasma membrane phosphatidylserine upon phagosome formation. LAP occurs in macrophages in various contexts, including in response to the phagocytosis of apoptotic cells (AC). RUBCN-deficiency impairs the degradation of engulfed AC. Herein, we show that, during efferocytosis, RUBCN depletion induces the stabilization of HIF-1 α , a hypoxia stress sensor known to regulate macrophage glycolytic metabolism. AC stimulation induces synthesis and stabilization of HIF-1 α in *Rubcn*^{-/-} bone marrow-derived macrophages (BMDM), as assessed by qPCR and immunoblot, respectively. The expression of glycolytic enzymes is increased in *Rubcn*^{-/-} BMDM in response to AC. We are currently investigating if HIF-1 α stabilization in the absence of RUBCN compromises tissue homeostasis. In a murine model with thymic cell death triggered by dexamethasone administration, we found by flow cytometry that myeloid RUBCN ablation (*Rubcn*^{flox/flox} LysM-Cre⁺ mice) leads to accumulation of necrotic cells (7-AAD⁺). Accumulation of necrotic cells was rescued in *Rubcn*^{flox/flox} *Hif1 α* ^{flox/flox} LysM-Cre⁺ mice, suggesting that HIF-1 α stabilization in the absence of RUBCN impairs continual efferocytosis. Using this model, we are currently performing histological analysis to evaluate cell death by TUNEL assay and determine HIF-1 α stabilization *in situ* by immunofluorescence microscopy. Thus, our preliminary findings suggest that HIF-1 α stabilization in the context of defective phagosome maturation due to defects in LAP affects macrophage capacity to perform efficient corpse clearance.

Keywords: Rubicon;LC3-associated phagocytosis;HIF-1 α .

3511 - Category: INNATE IMMUNITY (IN)

INVESTIGATION OF THE INFLUENCE OF CASPASE-8 IN THE CONTROL OF LEISHMANIA AMAZONENSIS INFECTION

DE MELO, D.K.C.; LOPES, C.D.A.; LIMA-JÚNIOR, D.D.S.; ZAMBONI, D.S.. USP, RIBEIRÃO PRETO - SP - BRASIL.

Leishmaniasis is a disease caused by the protozoan *Leishmania* spp., considered a significant global health challenge. Macrophage-mediated innate immunity is essential for the initial recognition of the pathogen. A key component of this mechanism is the NLRP3 inflammasome, which triggers the activation of caspase-1/11, assembling into a multiprotein platform that drives pyroptosis, a pro-inflammatory cell death pathway vital for parasite clearance. Traditionally described as an initiator caspase involved in apoptotic cell death, caspase-8 has been shown to be important in controlling pathogen replication in macrophages infected by *Legionella* and *Yersinia*, and has been associated with pyroptotic cell death. Here, we investigated the role of caspase-8 in pyroptosis-related inflammatory processes during *Leishmania amazonensis* infection of macrophages, using bone marrow-derived macrophages and molecular and immunological methods. Immunoblotting with a cleaved caspase-8-specific antibody, along with immunofluorescence microscopy, demonstrated that *L. amazonensis* infection induces caspase-8 activation in macrophages independently of canonical pyroptosis pathways. Moreover, flow cytometry of macrophages from Caspase-8/RipK3 double knockout mice revealed significantly increased susceptibility to *L. amazonensis*-RFP compared to wild-type controls after 24 hours of infection. Corroborating these *in vitro* findings, *in vivo* experiments showed that Ripk3/C1/C8^{-/-} mice exhibited heightened susceptibility to infection, displaying systemic dissemination of *Leishmania amazonensis*, a hallmark of severe disease progression. These findings suggest that caspase-8 is activated in response to *Leishmania* infection and is important for the parasite replication both in macrophages and *in vivo* as demonstrated through the infection of caspase-8-deficient mice with *Leishmania amazonensis*. Our study identifies caspase-8 as a potential target for novel therapeutic interventions.

Keywords: Leishmania;Inflamassome;Caspase-8.

2643 - Category: MOLECULAR IMMUNOLOGY (MI)

Impact of living in endemic areas on epigenetic aging and immunological profile

SILVA, C.G.¹; COELHO, M.M.¹; MOREIRA, F.C.¹; ZUCCHERATO, L.W.¹; TORRES, L.¹; VENTURA, L.H.D.A.¹; CAMATTA, G.C.¹; SOARES, B.F.S.¹; DURSO, D.F.¹; COSTA, M.S.¹; SATO, H.I.¹; TEIXEIRA, S.M.R.¹; TUPINAMBÁS, U.²; SILVEIRA-NUNES, G.³; MAIOLI, T.U.¹; FARIA, A.M.C.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, GOVERNADOR VALADARES - MG - BRASIL.

Introduction: Continuous exposure to antigenic stimuli of individuals living in endemic areas for infectious diseases stimulates the innate immune response and can accelerate biological senescence, measured by epigenetic clocks composed of DNA methylated CpG islands. Since this chronic exposure can impact immunosenescence, we aimed to compare biological aging and its association with the immunological profile of residents of endemic areas (EA) and non-endemic areas (NEA) in Brazil. **Methods:** Recruitment of 214 volunteers over 20 years of age in hospitals in the cities of EA (Governador Valadares/MG) and NEA (Belo Horizonte/MG) from December 2020 to October 2021. Peripheral blood samples were used for extraction of genomic DNA, and samples were loaded on the Illumina Infinium Methylation EPIC Bead Chip for methylation analysis. Analysis of blood mediators were performed by magnetic immunoassay in Luminex. Statistical analyses were performed in R. **Results:** EA residents showed accelerated aging compared to NEA residents in the six evaluated epigenetic clocks (skinHovarth, PedBE, Wu, TL, BLUP and EN clocks). EA residents showed higher production of IL-12p70, IL-17A and IL-9 as well as a less balanced serum mediator profile. NEA residents presented higher production of IL-6, IL-1B, IL-2, IL-1ra and IL-10. **Conclusion:** Chronic exposure to infectious agents results in accelerated biological aging and a disbalanced pro-inflammatory immunological profile.

Keywords: Immunosenescence;Epigenetic aging;Endemic area.

2647 - Category: MOLECULAR IMMUNOLOGY (MI)

Systems Immunology in Obesity: Single-Cell RNA-Seq Reveals Novel Macrophage-Specific Drivers of Chronic Inflammation

MOREIRA, F.C.¹; NAKAYA, H.I.²; MAIOLI, T.U.³. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS - UFMG, BELO HORIZONTE - MG - BRASIL; 2. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Obesity is marked by chronic low-grade inflammation, with macrophages in adipose tissue acting as key mediators of immune dysfunction. Macrophages play a crucial role in this process, undergoing significant gene expression changes. Understanding the molecular mechanisms driving these changes is critical for developing effective therapeutic strategies, yet the transcriptional regulators driving this immunometabolic reprogramming remain poorly understood. To better understand the molecular drivers of macrophage dysregulation in obesity, we applied a systems biology approach focused on immune gene networks. We combined bulk RNA-seq network analysis (WGCNA) with cross-species single-cell validation to identify conserved immune regulators in ATMs. Using murine and human scRNA-seq datasets (GEO), we mapped co-expression networks in obese versus lean conditions, prioritizing hub genes by betweenness centrality and participation coefficient. Our analysis recovered known obesity-associated immune regulators like *Stmn1*, *Cadm1*, *Asph*, *Myo1e*, *Sms*, *Pianp*, *Ndufs5*, *Ftl1*, *18002717Rik* and uncovered four novel hub genes *Gcsh*, *Rcan1*, *Ndufb4*, and *Ywhaq* that were not previously linked to macrophage biology or obesity. These novel genes displayed strong network connectivity and were robustly expressed in macrophage populations across species, suggesting a conserved role in macrophage mediated inflammation. Our integrative approach underscores the utility of combining co-expression network analysis with cross-species single-cell validation to uncover novel immune regulators in metabolic disease contexts both in mice and human cells. The identification of novel hub genes, provides valuable insights into the molecular mechanisms underlying macrophage dysfunction in obesity and suggests potential therapeutic targets for combating this complex disease.

Keywords: Obesity; Macrophages; Single-cell.

2655 - Category: MOLECULAR IMMUNOLOGY (MI)

Generation and Phenotypic Analyses of *Leishmania infantum* and *Leishmania amazonensis* Knockouts for the Miltefosine Transporter Gene

TATIANE, .¹; DOS SANTOS, L.M.¹; SUMAIA, P.A.¹; NASCIMENTO, F.C.¹; RICOTTA, T.Q.N.¹; BRAZIL, C.R.F.¹; RIBEIRO, I.S.¹; GRAZIELLE, V.²; CALDAS, G.D.A.B.²; TEIXEIRA, S.M.R.²; FERNANDES, A.P.². 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. CTIVACINAS, BELO HORIZONTE - MG - BRASIL.

Miltefosine (MIL), a phospholipid derivative initially used in cancer treatment, was later approved as the first oral drug for leishmaniasis. However, the emergence of MIL resistance in *Leishmania* spp. threatens its efficacy. MIL enters the cytoplasm through a phospholipid/miltefosine transporter (MT) and its beta subunit, ROS3. In resistant parasites, this translocation is impaired due to mutations in the MT gene. Alternative resistance mechanisms include altered reactive oxygen species (ROS) production, phosphatidylserine (PS) exposure, and changes in virulence. In this study, *Leishmania infantum* and *L. amazonensis* MT gene knockouts (KO) were generated using CRISPR/Cas9. These KO parasites were evaluated for ROS production, PS exposure, macrophage infectivity, and metabolic alterations (via GC-MS in *L. amazonensis*). KO parasites showed high resistance to MIL: *L. infantum* KO grew at MIL concentrations 100 times higher (800 µM) than wild-type (WT), while *L. amazonensis* KO exhibited an IC₅₀ six times higher than WT. In the presence of menadione, *L. infantum* KO parasites produced less ROS than WT. Under MIL exposure, both WT and KO generated increased ROS, with KO producing more ROS on the 7th day. *L. amazonensis* KO and WT produced similar ROS levels with menadione; however, KO parasites generated more ROS in the absence of MIL and even more when exposed to it, compared to unexposed KO. KO parasites from both species showed increased PS exposure in the presence of MIL but were less infective to macrophages than WT. More pronounced metabolic changes were observed in MIL-exposed WT parasites compared to KO, involving fatty acid, amino acid metabolism, and redox potential regulation. These findings confirm the role of the MT gene in MIL resistance and suggest that associated phenotypic changes are pleiotropic and influenced by parasite selection and species-specific metabolic background.

Keywords: *Leishmania* spp.; Miltefosine Transporter; gene knockout.

2732 - Category: MOLECULAR IMMUNOLOGY (MI)

Investigating the Immunomodulatory Potential of Recombinant HSP90 in Monocytic Cell Differentiation

ROCHA, I.; RAMOS, C.H.I.. UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS - SP - BRASIL.

Heat Shock Proteins (HSPs) are essential molecular chaperones that help maintain cellular proteostasis by facilitating protein folding, preventing aggregation, and supporting cellular homeostasis under stress conditions. While their intracellular roles are well established, emerging evidence indicates that HSPs also have immunoregulatory functions in the extracellular environment, influencing cytokine production and antigen presentation. For these effects to occur, HSPs must be secreted, and the literature provides supporting evidence for this secretion. However, the mechanisms by which extracellular HSPs interact with immune cells remain poorly understood. This study aims to model the activity of extracellular HSPs by evaluating the effects of recombinant HSPs on immune-related cell cultures, focusing on their potential role in immune modulation. At this stage, we are working on protein preparation. HSP90 was successfully expressed in *Escherichia coli* (DE3) and purified using nickel-affinity and size-exclusion chromatography, along with specific columns to remove immunogenic bacterial components. Human monocytic cells (THP-1) are being maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum under controlled conditions. Initial assays showed that the tested HSP induced the differentiation of these cells into macrophages. The next steps include testing its domains separately and designing experimental approaches such as ELISA, flow cytometry, confocal microscopy, metalomics, and metabolomics to evaluate their immunomodulatory effects and characterize the macrophage subtypes induced. By clarifying the extracellular roles of HSPs in immune regulation, this study may contribute to the development of new therapeutic strategies for inflammatory and immune-related diseases.

Keywords: Macrophages;Heat Shock Proteins;Differentiation.

2736 - Category: MOLECULAR IMMUNOLOGY (MI)

Immunomodulatory mechanisms of Extracellular Vesicles in pregnancies in situations of recurrent spontaneous abortion: A Scoping Review

GARRIDO, M.; MOREIRA, F.M.; RODRIGUES, A.F.F.; PANUCCI, L.A.G.; MORI, G.G.. UNIVERSIDADE DO OESTE PAULISTA (UNOESTE), PRESIDENTE PRUDENTE - SP - BRASIL.

Given the immune system's role in pregnancy, the immunomodulation generated by the use of extracellular vesicles (EVs) has been considered a possible treatment in situations of recurrent spontaneous abortion (RSA). Thus, this study evaluated, through a scoping review, the immunological mechanisms of EVs administered in cases of RSA in vivo, as well as their therapeutic applicability. This study was based on the Guidance for Conducting Systematic Scoping Reviews and was registered on the OSF Home platform. The systematic search was performed in the PubMed/MEDLINE, Scopus, and Cochrane Library databases up to June 2025 by two independent reviewers to answer the question: Would EVs modulate the process of RSA? The primary outcomes were the molecular pathways involved in the control of RSA. The risk of bias was analyzed according to the criteria of SYRCLE's RoB tool. A total of 440 articles were analyzed, and after applying the eligibility criteria and excluding duplicates, 3 studies were selected. All studies showed a significant reduction in embryo resorption rate in animals. Bone marrow stem cell-derived EVs modified the lymphocyte pattern to Th2, activated M2 macrophages, and reduced the number of CD8+ T lymphocytes in the uterine microenvironment, as well as contributed to an overexpression of IL-4 and IL-10, supporting the maintenance of pregnancy. Decidual stem cell-derived EVs inhibited the expression of B-TrCP, increasing the levels of Snail protein and decreasing the expression of E-cadherin, which contributed to the epithelial-mesenchymal transition during implantation. Trophoblast-derived EVs from normal pregnancies carried HLA-E to NK cells in the uterine environment, reduced the Th1 lymphocyte pattern, and released VEGF-alpha, contributing to angiogenesis and trophoblast growth. Most studies showed an unclear risk of bias. It was concluded that EVs have immunomodulatory relevance in pregnancy and potential therapeutic applicability. FAPESP 2024/05027-9

Keywords: Extracellular vesicles;Immunomodulation;Recurrent Spontaneous Abortion.

2749 - Category: MOLECULAR IMMUNOLOGY (MI)

Use of extracellular vesicles as biomarkers for the diagnosis of Rheumatoid Arthritis: Systematic Review

DE ALMEIDA, J.A.; DOMINGUES, V.T.; DE SÁ, F.M.C.; RODRIGUES, A.F.F.; MORI, G.G.. UNOESTE, PRESIDENTE PRUDENTE - SP - BRASIL.

Rheumatoid arthritis (RA) is a multifactorial autoimmune inflammatory disease with a polyarticular pattern and systemic repercussions. Early diagnosis is essential to improve the lifespan and survival of patients. Extracellular vesicles (EVs) have emerged as a promising alternative biomarker. Thus, the objective of this study was to evaluate the effectiveness of EVs as a biomarker for the diagnosis of RA. This systematic review followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). A systematic search was conducted in the PubMed/MEDLINE, Scopus, and Cochrane Library databases up to March 2025 to answer the PICO question: "Would the use of EVs as a biomarker be more effective than currently used markers for the diagnosis of RA?" Diagnostic effectiveness was considered the primary outcome. Risk of bias analysis was performed using the QUADAS-2 tool. A total of 1,521 scientific articles were analyzed, and after applying eligibility criteria, excluding duplicates, and performing full-text reading, 12 studies were included in the review. All studies inferred the potential of EVs as biomarkers for RA. Different miRNAs and molecules of EVs can be biomarked, evidencing a variety of specificity and sensitivity indices. Eight studies presented diagnostic accuracy, with a mean of 0.834 ± 0.120 . The studies indicated the greater efficiency when molecules of EVs were biomarked in combination. Two studies provided a quantitative comparison with the currently used biomarker; in one of them, all analyzed EVs showed greater accuracy, while in the other, only the combined EVs presented superior accuracy. The risk of bias analysis was found to be uncertain for most of the assessed domains. It was concluded that the use of EVs can contribute to the diagnosis of RA. However, more clinical studies comparing their effectiveness with current biomarkers are necessary for a more in-depth analysis. FAPESP Processo 2024/18695-0.

Keywords: Diagnosis;Exosome;Rheumatoid Arthritis.

2839 - Category: MOLECULAR IMMUNOLOGY (MI)

Alterations in the Cytokine Profile of Neutrophils in an Ischemia-Reperfusion Model

PEREIRA, W.G.F.; GODOI, E.A.; RIBEIRO, D.G.; ARAÚJO, R.K.S.; CASTRO, M.D.S.; FONTES, W.. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

Ischemia-reperfusion (I/R) injury remains a significant clinical challenge, often leading to systemic dysfunctions and high mortality. Neutrophils are key mediators in this process by releasing cytokines and reactive oxygen species that amplify inflammation. Ischemic Preconditioning (IPC) has emerged as a promising intervention, exerting immunomodulatory effects that mitigate I/R induced tissue damage.

This study investigated the cytokine profile produced by neutrophils under different experimental conditions, to evaluate the protective effects of IPC. Twenty Wistar rats were assigned to four experimental groups: Control (CTRL), Laparotomy (LAP), I/R and IPC. After the respective surgical procedures, blood samples were collected for neutrophil isolation and cytokine profiling using a Luminex multiplex assay.

The I/R group showed increased levels of pro-inflammatory cytokines, particularly CCL-5 and CXCL-2, indicating amplification of the inflammatory response and leukocyte recruitment. In contrast, the IPC group exhibited increased levels of IL-12p70 and G-CSF, cytokines associated with immunomodulation and activation of anti-apoptotic pathways such as PI3K-AKT-BCL2, these pathways may protect "anti-inflammatory" neutrophils, thereby minimizing tissue damage. Notably, IFN- γ was upregulated in the IPC group, suggesting preserved immune functionality and a possible neuroprotective role. The regulated expression of CCL-2 in IPC reinforces the hypothesis of a controlled inflammatory environment, compatible with regenerative effects.

These findings suggest that IPC not only reduces inflammatory damage but modulates the neutrophil function, mitigating the damage caused by prolonged ischemia and promoting a protective immunological environment in the ischemia-reperfusion process. These findings advance our understanding of the underlying immunological mechanisms and identify promising targets for postconditioning therapeutic strategies.

Keywords: Ischemia and reperfusion;neutrophils;cytokines.

2884 - Category: MOLECULAR IMMUNOLOGY (MI)

Potential use of the recombinant allergen Poly p 5 derived from the venom of *Polybia paulista* wasp for evaluating specific Immunoglobulin E in patients submitted to venom immunotherapy

CHAGAS, J.D.A.; MINATI, L.S.; ZOLLNER, R.D.L.; FERNANDES, L.G.R.. STATE UNIVERSITY OF CAMPINAS, CAMPINAS - SP - BRASIL.

Introduction: Venom immunotherapy (VIT) is the only proven approach to prevent severe allergic reactions to stings and provide long-term benefits. In Brazil, the social wasp *Polybia paulista* is clinically relevant. Measuring specific IgE (sIgE) to venom allergens is essential for diagnosis and VIT monitoring. However, current tests often use crude or recombinant allergens from wasps of other regions, potentially reducing accuracy. **Objectives:** We evaluated the role of recombinant allergen antigen 5 (Poly p 5) from *P. paulista* wasp in developing indirect ELISA tests for quantifying sIgE levels in serum samples from allergic patients undergoing VIT protocol. **Methodology:** Sera from allergic patients (n=11) previously submitted to VIT protocol were obtained from the Laboratory of Translational Immunology biobank. Patients were diagnosed based on clinical history, skin prick tests, and sIgE values in ImmunoCAP test to allergen i3 (common wasp venom >0.3kU/L). Ethics committee approval was obtained (license 80822817.3.0000.5404). Serum samples collected at protocol initiation, after 6 months, and end of VIT were used to detect sIgE to crude *P. paulista* venom allergens and rPoly p 5 by indirect ELISA. Crude venom allergens were obtained from venom glands and semi-purified by ultrafiltration using 3kDa membranes. Recombinant Poly p 5 was produced by heterologous expression in *Pichia pastoris* yeast. Data were analyzed using Wilcoxon test, Spearman correlation, and Bland-Altman tests ($p < 0.05$). **Results:** Greater differences were observed between crude wasp venom-based ELISA compared to rPoly p 5 at protocol initiation ($p = 0.042$); however, they converged over time, showing high correlation ($r = 0.76$) at immunotherapy completion with no statistical difference ($p = 0.123$), suggesting similarity between quantification methods. **Conclusions:** Recombinant allergens derived from major Hymenoptera venom allergens may serve as potential tools for assessing sIgE in VIT outcomes. **Support:** FAPESP.

Keywords: Hymenoptera venom allergy; Venom immunotherapy; Recombinant allergens.

2915 - Category: MOLECULAR IMMUNOLOGY (MI)

CHARACTERIZATION OF GADD45 EXPRESSION DURING CD4+ T CELL ACTIVATION AND DIFFERENTIATION

MAXIMO, M.L.D.S.; BORONI, M.; VIEIRA, B.L.M.D.O.; VIOLA, J.P.D.B.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

CD4 T cells are immune cells whose main activity is the secretion of cytokines to orchestrate the immune response. For CD4 T cells activation and differentiation are necessary: the antigen stimulation by TCR recognition, co-stimulus by CD28 receptor, and the cytokines signaling in the microenvironment. To better understand the molecular mechanisms regulating T cell activation at an early time point, our group has performed an RNA-seq with activated CD4 T cells for 1 hour. Among the upregulated genes, was GADD45 β (Growth Arrest and DNA Damage inducible β), a member of the GADD45 family, which has different activities, including the activation and differentiation of immune cells. During T cell activation, GADD45 α is known to silence ZAP-70 mediated p38 alternative activation, which is important for proper T cell function. On the other hand, GADD45 β and γ activate the MAPK cascade which classically activates p38. The role of classically activated p38 remains controversial regarding T cell activation. Thus, the current project aims to characterize expression of GADD45 genes during activation and after differentiation to Th1, Th2 and Th17. Therefore, primary CD4 T cells derived from C57Bl/6 mice were activated with anti-CD3 and anti-CD28 and skewed toward the desired phenotypes. We then analyzed GADD45 expression via qRT-PCR, which demonstrated that GADD45 α was downregulated from 4h to 72h after activation. On the other hand, GADD45 β was upregulated at 4h but it was then downregulated, maintaining basal levels from 24h to 72h after activation. GADD45 γ was downregulated at 24h post activation, but was notably upregulated from 48h until 72h, when it reached its peak. Regarding polarization, differential expression among phenotypes was not observed for any GADD45 gene. Then, we conclude that the GADD45 genes are differentially expressed during T cell activation.

Keywords: CD4 T Cell; GADD45; T Cell activation.

2942 - Category: MOLECULAR IMMUNOLOGY (MI)

Investigation of the regulatory mechanisms of the IRF2BP2 cofactor over CD4⁺ T cell activation and differentiation

TAN, L.B.; VIEIRA, B.L.M.D.O.; VIOLA, J.P.D.B.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

IRF2BP2 is a transcriptional cofactor capable of interacting with a variety of transcription factors (TF) and participating in the regulation of their target genes, including some that are relevant in the context of CD4⁺ T cell activation and differentiation such as NFAT, IRF2 and KLF2. Independent groups have described mutant variants of IRF2BP2 in humans that are associated with immunological disorders, such as deficiency and/or disfunction of B and T cells, as well as the promotion of Acute Lymphoid Leukemia. We showed that IRF2BP2^{fl/fl} Lck-Cre mice, which have high IRF2BP2 expression in T cells, present with a sharp reduction of lymph node and spleen size, lower amounts of CD4⁺ T helper cells and an increase in Treg cells. Purified CD4⁺ T cells from these animals activated *ex vivo* have deficient clonal expansion and are strongly skewed towards the Th1 phenotype, assessed by interferon- γ production and T-bet expression, as early as 24 hours after activation. This happens despite the presence of inhibitory polarizing cytokines during activation, such as IL-4. Thus, IRF2BP2 seem to play an important role in CD4⁺ T cell biology, from ontogeny to activation and differentiation, however the molecular mechanisms involved in this process are still unknown. We seek to identify the IRF2BP2 regulated genes and their roles in promoting the observed changes in CD4⁺ T cell biology, for which we will employ transcriptomic analysis and the silencing of identified target genes. In recent years the relevance of CD4⁺ T cells in cancer immunity has gained increasing recognition, particularly in the context of immunotherapy. CD4⁺ T cells are necessary to generate and sustain cytotoxic CD8⁺ T cells, provide help to the innate immunity and may possess cytotoxic activity to induce tumor cell death directly. Thus, we will also investigate the role of IRF2BP2 in CD4⁺ T cells during the immune response to murine tumors with the potential for identification of new molecular targets.

Keywords: CD4⁺ T cells;IRF2BP2;Cancer immunology.

2984 - Category: MOLECULAR IMMUNOLOGY (MI)

Production of Human Monoclonal Antibodies Against Dengue Virus with Modified Fc Portions for Enhanced Stability

OTÁLORA, N.A.; AZEVEDO, I.R.; SPECKHORT, D.; SILVA, G.A.D.S.; YAMAMOTO, M.M.; ANTONIO, N.D.S.; ALMEIDA, B.D.S.; BOSCARDIN, S.B.. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

INTRODUCTION: Dengue, caused by the DENV virus and transmitted by *Aedes aegypti*, affects over 100 countries. This major public health issue leads to approximately 400 million annual infections. Projections indicate the at-risk population will grow from 53% (2015) to 63% (2080) due to climate change and urbanization. Despite existing vaccines, their limited efficacy against all four DENV serotypes necessitates alternative therapies. Our group previously developed four human monoclonal antibodies (mAbs)—A3D, C4D, D2D, and F11D—that target the viral envelope (E) protein.

AIM: To produce the four anti-DENV mAbs, with and without Fc portion mutations, to reduce Fc γ receptor binding and extend mAb half-life in circulation.

METHODS AND RESULTS: Plasmids encoding the heavy and light chains of mAbs A3D, C4D, D2D, and F11D were transiently transfected into Expi293TM cells. Produced mAbs were purified from cell culture supernatants and integrity was confirmed by SDS-PAGE. ELISA against all four DENV serotype E proteins demonstrated differential reactivity. Neutralization assays were performed against DENV-2 and ZIKV infection. While the mAbs showed no neutralization capacity against ZIKV, all successfully neutralized DENV-2 infection, exhibiting varying IC50 values.

Concurrently, variable region DNA sequences of mAb heavy chains were cloned into three plasmids with specific Fc mutations: LALA (to decrease Fc γ binding), LS (to enhance pH-dependent human FcRn binding), and a LALA/LS combination. These modified mAbs are expressing.

CONCLUSIONS: We successfully produced four human anti-DENV mAbs demonstrating neutralization capacity against DENV-2 infection. Future work includes testing their neutralization against DENV-1, -3, -4, and evaluating Fc-mutated mAbs for reduced Fc γ binding and extended half-life in circulation.

This work is supported by FAPESP (#2024/01667-3); CNPq (#307596/2023-0) and CAPES (finance code 001)

Keywords: Dengue;monoclonal antibodies;envelope protein.

3007 - Category: MOLECULAR IMMUNOLOGY (MI)

Development of Monoclonal Antibodies Against the Infectious Forms of *Plasmodium vivax*

SILVA, M.D.O.¹; FERREIRA, N.S.²; WANG, Z.³; FUMAGALLI, M.J.³; GAZUMYAN, A.³; HERNANDEZ, B.³; YAMAMOTO, M.M.²; DE OLIVEIRA, T.R.²; SOARES, I.D.S.²; NUSSENZWEIG, M.C.³; BOSCARDIN, S.B.². 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. THE ROCKEFELLER UNIVERSITY, NEW YORK - ESTADOS UNIDOS DA AMERICA.

Plasmodium vivax is the most prevalent malaria-causing species outside the African continent. While less fatal than *P. falciparum*, it contributes substantially to malaria-related illness and can lead to severe complications. To date, there is no available vaccine against this species, and solutions for prevention and treatment of infection are extremely relevant. Human monoclonal antibodies (hmAbs) targeting the circumsporozoite protein (CSP) have shown potential protective effects against *P. falciparum*. However, the development of such antibodies specifically against *P. vivax* has been less explored. In this study, we report the isolation of human monoclonal antibodies specific to the *P. vivax* CSP (PvCSP) from individuals naturally infected in an endemic region of Brazil. Memory B cells were sorted using a hybrid PvCSP protein containing repeat domains from the three PvCSP variants (VK210, VK247, and *P. vivax*-like), collectively referred to as PvCSP-all epitopes. A total of 80 paired heavy and light chain antibody sequences were obtained from six donors. Sequence analysis revealed expanded clones of CSP-specific memory B cells in almost all individuals. V gene analysis showed preferential usage of certain gene segments, with a notable overexpression of IGHV3-33, IGLV3-10, and IGKV2-28 family genes. Thirty-eight representative antibody sequences were selected for expression and further characterization. ELISA-based binding assays showed that nearly all antibodies bound to the PvCSP-All- epitopes construct, with most of them displaying cross-reactivity to multiple variants and some showing allele-specific recognition. Although these antibodies will still be further evaluated for their neutralizing capacity in vitro and in vivo, they already demonstrate potential as valuable tools for antibody-based interventions and may contribute to reducing the global burden of *P. vivax* malaria.

Keywords: Malaria; monoclonal antibodies; Memory B cells.

3023 - Category: MOLECULAR IMMUNOLOGY (MI)

PEPTIBODIES: A LOW-COST ALTERNATIVE TO ANTI-PD-L1 MONOCLONAL ANTIBODIES

NUNES, J.E.S.¹; BARTH JUNIOR, V.C.²; BONORINO, C.¹. 1. UFCSPA, PORTO ALEGRE - RS - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL.

The success of immune checkpoints immunotherapy has recently spurred significant efforts in the development of new drugs. In particular, blocking PD-1/PD-L1 interactions using monoclonal antibodies (mAbs) restores T-lymphocyte activity and enables the immune system to control tumors. Currently, anti-PD-1 mAb treatments require prior assessment of PD-L1 expression in tumor tissue, typically performed by immunohistochemistry (IHC) using an anti-PD-L1 mAb. Therefore, PD-L1 is a strategic target both for immune checkpoint blockade and as a biomarker guiding treatment decisions with anti-PD-1 mAbs. However, the high cost of production limits universal access, particularly via Brazil's public health system (SUS). As an alternative, peptibodies combine the specificity of bioactive peptides with the pharmacokinetic and cytotoxic properties of antibodies, achieved through fusion of these peptides with the Fc region of human antibodies. Peptibodies can be produced in bacteria at substantially lower cost than mammalian cell expression systems. We designed one such molecule targeting PD-L1 with potential applications in cancer treatment and PD-L1 diagnosis via IHC. Peptide sequences with known PD-L1 binding capacity were identified from the literature and selected for peptibody design. One sequence was synthesized via overlap-extension PCR and cloned into the pET30a(+) vector. It was recombinantly expressed in SHuffle T7 (DE3) bacterial strain and expression conditions were determined. The soluble fraction from bacterial cytosol was submitted to affinity chromatography for peptibody isolation. Binding to PD-L1 and competition with PD-1 were confirmed by ELISA and SPR. T-lymphocyte activity recovery was assessed by co-culture with PD-L1 expressing cells incubated with and without peptibody. Our results indicate this can be a viable alternative to monoclonals targeting PD-L1.

Keywords: Immunotherapy; Peptibodies; Immune Checkpoint Blockade.

3027 - Category: MOLECULAR IMMUNOLOGY (MI)

EROS controls NT-GSDMD pyroptosis and IL-1 secretion in macrophages

DA SILVA, G.R.¹; LIANG, Y.²; RUBIO, G.G.²; RIVERA, K.²; VIEIRA, P.M.M.D.M.¹; EVAVOLD, C.². 1. STATE UNIVERSITY OF CAMPINAS, CAMPINAS - SP - BRASIL; 2. RAGON INSTITUTE OF MASS GENERAL, MIT, AND HARVARD, CAMBRIDGE - ESTADOS UNIDOS DA AMERICA.

Inflammatory caspases cleave gasdermin D (GSDMD) to release a pore-forming N-terminal fragment (NT-GSDMD) that mediates pyroptosis by permeabilizing the plasma membrane and secreting IL-1 family cytokines. While upstream regulation of GSDMD cleavage via inflammasomes is well characterized, downstream control of NT-GSDMD pore formation is less understood. Using CRISPR genetic screening in Cas9-expressing murine immortalized bone marrow derived macrophages (iBMDMs) with inducible expression of NT-GSDMD, we identify essential for reactive oxygen species (EROS) as a novel regulator of pore formation. EROS deficiency in macrophages impairs NT-GSDMD oligomerization, membrane permeabilization, and membrane rupture. Although EROS stabilizes NADPH-oxidase (NOX) subunits for phagosomal ROS, NOX activity is dispensable for pyroptosis in this context, suggesting alternative ROS sources are active during inflammasome signaling such as from metabolic pathways and mitochondria. EROS is also necessary for membrane permeability and IL-1 β secretion following NLRP3 activation via bacterial lipopolysaccharide and Nigericin (LPS/N) treatment. Mechanistically, we confirm recent reports that EROS acts as a chaperone for P2 receptor X7 (P2RX7) to mediate protein stability. Notably, P2RX7 sgRNAs are also enriched within survivors from our CRISPR/Cas9 screen, and we validate that P2RX7 deficiency results in reduced membrane permeability after NT-GSDMD expression in macrophages. Together, our findings suggest a novel regulatory pathway for NT-GSDMD-driven pyroptosis controlled by EROS. Whether EROS regulates additional cargos to control pyroptosis beyond P2RX7 is the subject of our ongoing work.

Keywords: Pyroptosis;EROS;P2RX7.

3033 - Category: MOLECULAR IMMUNOLOGY (MI)

Engineering BoLA-DR monoallelic cell lines to advance CD4 T-cell epitope prediction through immunopeptidomics and machine learning

DE MORAES, A.¹; FISCH, A.²; NILSSON, J.B.³; TERNETTE, N.⁴; NIELSEN, M.³; CONNELLEY, T.²; FERREIRA, B.R.¹. 1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. THE ROSLIN INSTITUTE, THE UNIVERSITY OF EDINBURGH, EDINBURGH - REINO UNIDO; 3. TECHNICAL UNIVERSITY OF DENMARK, LYNGBY - DINAMARCA; 4. UNIVERSITY OF DUNDEE, DUNDEE - REINO UNIDO.

Accurate prediction of peptides presented by specific MHC alleles is essential for identifying T-cell epitopes, a critical step in vaccine and immunotherapy development. In veterinary immunology, significant advances have been achieved with the creation of NetBoLApan – an MHC ligand prediction tool for Bovine Leukocyte Antigen (BoLA) – trained on BoLA-eluted ligand data obtained through advanced mass spectrometry peptidomics (immunopeptidome). To enhance NetBoLApan's molecular coverage, we generated BoLA-DR monoallelic bovine cell lines using alleles from economically relevant cattle breeds such as Holstein-Friesian, Nelore, and Girolando. A retrospective analysis revealed ten BoLA-DR alleles prevalent in South American and British herds that lacked immunopeptidomics data. Incorporating these alleles would extend NetBoLApan's coverage to over 90% of these breeds. Full-length BoLA-DRB3 sequences were amplified by RT-PCR, cloned into SCRPSY lentiviral vectors, and transfected into HEK293FT cells to produce lentiviral particles. These were used to deliver BoLA-DR alleles into BoLA-I/DR knock-out MDBK cells, generating monoallelic bovine cell lines. Expression was induced by IFN-gamma and validated by flow cytometry. The immunopeptidome were characterized by LC-MS/MS and used to train allele-specific prediction models using the NNAlign framework. These models revealed clear MHC-II binding motifs and high predictive performance (AUC 0.77–0.90). In conclusion, engineered BoLA-DR monoallelic bovine cell lines represent a powerful platform for immunopeptidomics and machine learning-driven epitope prediction, enhancing CD4 T-cell antigen discovery in cattle.

Keywords: MHC;Immunopeptidomics;Epitope prediction.

3053 - Category: MOLECULAR IMMUNOLOGY (MI)

OPTIMIZING MONOCLONAL ANTIBODY SURFACE DISPLAY AND DELIVERY VIA *SACCHAROMYCES BOULARDII* FOR ORAL BIOTHERAPEUTIC APPLICATION

GUIMARÃES, P.O.; PINHATE, S.B.; DE MATOS, A.L.T.; RIBEIRO, I.B.D.O.; MARANHÃO, A.Q.; BRIGIDO, M.D.M.. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

Anti-CD3 antibodies are promising candidates for oral tolerance-based therapies, and *Saccharomyces boulardii*, a probiotic yeast, offers a suitable delivery vehicle. This study evaluated *S. boulardii* for surface display of an anti-CD3 in a single-chain Fv format (scFv). Three integrative vectors were constructed, each containing GFP, G418 resistance, and different retroelement targeting sequences (Ty1.2, Ty2, and Ty2mp) to induce homologous recombination. Among them, Ty2mp exhibited the highest transformation efficiency—nine times greater than Ty2—while Ty1.2 failed to generate viable clones. A Ty2mp-based vector was then used to express the anti-CD3 scFv. Compared to an episomal vector (AGA) encoding the same antibody fragment, the Ty2mp-based vector showed 3.5 times higher transformation efficiency and improved expression, with 80% of positive cells compared to only 40% for the episomal vector, as assessed by flow cytometry. Antibody display was enhanced at 25°C, reaching up to twofold the expression observed at 30°C. Stability assays revealed that only Ty2mp clones retained antibiotic resistance after five generations in non-selective medium, followed by re-exposure to selective plates, compared to AGA. To improve oral delivery due to harsh gastrointestinal conditions, we developed a DOPA:cholesterol lipid capsule, achieving 97% encapsulation efficiency. Encapsulated yeast showed improved mean survival after three hours of incubation in simulated intestinal fluid at 37°C (75.04%) versus non-encapsulated cells (55.85%). However, in simulated gastric fluid, non-encapsulated cells performed slightly better (44.8% vs. 38.4%). In conclusion, Ty2mp outperformed in all tested parameters: transformation efficiency, expression, and stability. The lipid capsule enhanced yeast survival under intestinal conditions. Together, these results validate encapsulated *S. boulardii* as a promising vehicle for oral therapy delivery, warranting further evaluation in disease models.

Keywords: Anti-CD3;Biotherapy;Oral delivery.

3056 - Category: MOLECULAR IMMUNOLOGY (MI)

The role of recombinant allergens Poly p 1 and Poly p 5 derived from *Polybia paulista* wasp venom in experimental model of wasp venom desensitization

MINATI, L.S.; CHAGAS, J.D.A.; ZOLLNER, R.D.L.; FERNANDES, L.G.R.. UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS - SP - BRASIL.

Introduction: *P. paulista* wasp causes sting accidents and anaphylactic events in Brazil. Venom desensitization using recombinant allergens represents a promising immunotherapeutic strategy, with enhanced safety and specificity. However, tolerance induction mechanisms using recombinant allergens require further investigation. Objectives: We evaluated the role of rPoly p 1 and rPoly p 5 derived from *P. paulista* wasp venom in an experimental desensitization model by assessing specific immunoglobulins (slg) and regulatory cell profiles. Methodology: Venom allergens were obtained from *P. paulista* venom glands and semi-purified by ultrafiltration. Recombinant Poly p 1 and Poly p 5 were produced in *E. coli* and *Pichia pastoris*, respectively. Female BALB/c mice (n=10, ethics approval 5732-1/2021) were immunized with 6 weekly intradermal (i.d.) doses of 20µg *P. paulista* protein extract. After 7 days, mice sera were obtained. After 14 days, mice were divided into treated and control groups (n=5 each). The treated group received 3 i.d. of rPoly p 1 and rPoly p 5 (20µg each); controls received saline. Twenty days post treatment, mice were challenged intraperitoneally with 20µg venom extract, sacrificed after 30 minutes, and sera/splenic cells collected. Cells were stimulated in vitro with 3µg/ml venom extract for 96 hours, followed by flow cytometry analysis for T and B regulatory cell markers. ELISA assessed slgE, slgG1, and mouse mast cell protease-1 (mMCPT-1). Results: rPoly p 1 and rPoly p 5 treatment reduced slgE and slgG1 in sensitized mice. Treated mice showed decreased mMCPT-1 concentrations and increased Foxp3+Helios+ Tregs, with PD-L1 overexpression in CD19+CD5+CD1d+ Bregs following antigenic challenge compared to controls. Conclusions: rPoly p 1 and rPoly p 5 allergens in desensitization protocols represent a promising strategy to modulate wasp venom-driven allergic immune responses, reducing slgs and enhancing peripheral regulatory cell activity. Support: FAPESP

Keywords: Hymenoptera venom allergy;venom immunotherapy; recombinant allergens.

3103 - Category: MOLECULAR IMMUNOLOGY (MI)

Expression of Human Monoclonal Antibodies Anti-ZIKV in Mammalian Cells in FvFc format

PINHEIRO, M.C.M.¹; RODRIGUES, L.S.¹; BARBOSA, A.C.A.¹; BEZERRA, M.R.L.²; FRANÇA, R.K.A.D.O.¹; BRIGIDO, M.D.M.¹; MARANHÃO, A.Q.¹. 1. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, EUSÉBIO - CE - BRASIL.

Flavivirus infections, especially by Dengue, Zika and Yellow Fever virus, have major impact in worldwide health, particularly in Brazil. Due to the high number of cases and the complexity of the clinical manifestations of hemorrhagic, neurological syndrome and congenital microcephaly, it is crucial to develop ways of preventing and ameliorating these disease symptoms. An alternative is the development of neutralizing antibodies that could be used to prevent virus effects during pregnancy. Thus, human monoclonal antibodies specific to the envelope fusion peptide, a conserved epitope among flavivirus, were selected by Phage Display technique. This work aims to express these antibodies in a heterologous system of mammalian cells in FvFc (scFv fused to IgG1 Fc) format. CHOK1 and Expi293 cells were transfected with plasmid encoding FvFc sequence, in tandem with GFP (Green Fluorescent Protein) gene and a Puromycin resistance gene, intercalated by IRES sequences in a tricistronic way. Transfection efficiency was analyzed based on the percentage of GFP-positive cells by flow cytometry, and transfected cells were sorted by GFP marker. Puromycin was used to select a stable pool of cells, and a limiting dilution was performed to establish the best antibody-producing clone. After expression, the culture supernatants were purified by affinity chromatography in Protein A column. The recombinant proteins were analyzed by SDS-PAGE and Western Blotting and binding to ZIKV and DENV4 was assessed by indirect ELISA. The transfected cells used presented a percentage of approximately 40% GFP-positive cells. The produced and purified protein was visualized by SDS-PAGE and Western blotting. The binding capacity of the AZ1p antibody to ZIKV and DENV4 was demonstrated. PRNT assays will be performed to determine the ZIKV neutralization ability of AZ1p.

Keywords: Flavivirus;Immunotherapy;Human Monoclonal Antibodies.

3107 - Category: MOLECULAR IMMUNOLOGY (MI)

Memory B cell repertoire reveals different isotypes sharing the same paratope after SARS-CoV-2 vaccination and infection

BARBOSA, B.C.C.¹; DL, O.¹; MAIA, L.C.R.¹; BECK, B.K.¹; BONOMO, A.²; VICTORA, G.¹; VALE, A.M.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

The SARS-CoV-2 pandemic was controlled through widespread immunization, which led to the generation of immunological memory. Upon antigen exposure, activated B cells form germinal centers, where they undergo somatic hypermutation and affinity maturation with T cell help. High-affinity B cells differentiate into plasma cells or memory B cells (MBCs). IgM⁺ B cells may also undergo class switch recombination, giving rise to other isotypes such as IgA, important for mucosal immunity, or IgG, which predominates in serum. Despite advances in our understanding of the SARS-CoV-2 humoral response, it remains unclear whether IgG⁺ and IgA⁺ MBCs share the same variable region repertoire and originate from the same clonal lineages. To address this, we analyzed the MBC immunoglobulin repertoire of two individuals who received four vaccine doses and subsequently tested positive for SARS-CoV-2. From blood samples, Spike-specific switched MBCs were single-cell sorted and cultured. Cells were harvested for RT-PCR, followed by Sanger sequencing and IMGT annotation of the immunoglobulin variable regions. A total of 115 clones were sequenced, of which 87% were IgG and 13% were IgA. On average, germline mismatch rates were similar between IgG (7%) and IgA (6.5%) clones. Notably, we identified one IgG and one IgA clone that shared the same paratope, indicating clonotype overlap despite distinct isotypes. Junction analysis revealed shared VH, DH, and JH genes, 80% identity in the CDR-H3 sequence, and 100% identity in the N-region additions. The IgG clone exhibited 20 VH mutations, while the IgA clone displayed 28, suggesting further diversification. These findings support a shared clonal origin and raise the possibility of sequential class switching from IgG to IgA during affinity maturation. Given the limited number of sequences, further high-throughput studies are needed to confirm this process. Such insights could inform strategies to enhance mucosal immunity through vaccination.

Keywords: SARS-CoV-2;Class switch recombination;Memory B cells.

3111 - Category: MOLECULAR IMMUNOLOGY (MI)

Regulation of SAA1 Expression in White Adipose Tissue: Insights from Metabolic and Sex-Related Contexts

SILVA, L.D.R.¹; GARCÍA, H.D.M.²; CAL, T.C.M.F.²; BORONI, M.³; VIEIRA, P.M.M.D.M.²; MORI, M.A.D.S.²; LEIRIA, L.O.S.¹. 1. SCHOOL OF MEDICINE OF RIBEIRÃO PRETO, UNIVERSITY OF SÃO PAULO (USP), RIBEIRÃO PRETO - SP - BRASIL; 2. INSTITUTE OF BIOLOGY, UNIVERSITY OF CAMPINAS (UNICAMP), CAMPINAS - SP - BRASIL; 3. NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

Obesity is a chronic and multifactorial inflammatory disease that affects approximately 16% of the adult population and leads to an increased risk of developing other conditions such as type 2 diabetes mellitus (T2DM) and cardiovascular diseases. Considered a metabolic disorder, its development involves the dysregulation of important hormones, such as insulin and leptin. Moreover, other soluble elements play a role in the pathophysiology of these diseases. Among these are serum amyloid A (SAA) proteins, which participate in inflammation and lipoprotein remodeling. The subtypes SAA1 and SAA2 stand out for exhibiting higher serum levels in patients with obesity. In this study, we sought to understand how the metabolic context of the organism influences the expression of SAA1 by white adipose tissue (WAT), whether or not associated with a metabolic disorder. Our *in silico* analyses were performed using a single-nucleus RNA sequencing dataset from biopsy samples of subcutaneous and visceral adipose tissue from adult humans, collected by biopsy and classified by biological sex, body mass index (BMI), and physiological state (healthy, diagnosed with obesity and/or T2DM). Interestingly, subcutaneous adipose tissue showed higher expression of SAA1 compared to visceral adipose tissue. A positive correlation with patients' BMI was also observed, with a progressive increase in expression from lean individuals to those overweight and obese. Diabetic patients showed higher levels of SAA1 compared to healthy individuals. Sexual dimorphism analysis demonstrated that women express more SAA1 in WAT than men, suggesting a possible hormonal influence on this regulatory pattern. As next steps, we aim to investigate the regulation of SAA1 in adipocytes via stimulation by sex hormones through *in vitro* assays. This study offers insights into the regulatory mechanisms of SAA1 in white adipose tissue and its possible relationship with the incidence and pathophysiology of metabolic diseases.

Keywords: Metabolic diseases;SAA1;Adipokines .

3119 - Category: MOLECULAR IMMUNOLOGY (MI)

IL-6 and OSM induce bone formation through activation of gp130 using Shc1 as an adapter protein to activate STAT3

DE SOUZA, P.P.C.¹; ACIOLI, G.M.D.R.¹; HENNING, P.M.²; DE ARAÚJO, M.C.¹; MARCELINO, T.F.³; NUNES, G.C.³; LERNER, U.H.⁴. 1. UNIVERSIDADE FEDERAL DE GOIÁS, GOIÂNIA - GO - BRASIL; 2. GOTHENBURG UNIVERSITY, GOTHENBURG - SUECIA; 3. UNIVERSIDADE ESTADUAL PAULISTA, ARARAQUARA - SP - BRASIL; 4. GOTHENBURG, GOTHENBURG - SUECIA.

Inflammatory processes close to the bone often lead to bone loss. The prevailing view that inflammation reduces bone mass through increased resorption and inhibition of bone formation has been challenged by observations of bone apposition near inflamed tissues. Members of the IL-6 cytokine family signal through the shared gp130 receptor and are involved in bone homeostasis. Single-cell RNA sequencing of calvarial osteoblasts differentiated for 11 days in osteogenic medium revealed expression of *Il6st* (gp130), *Lifr*, and *Osmr*, indicating the presence of key receptors for IL-6 family cytokines. Functional assays revealed that among the tested cytokines, only IL-6 (in combination with its soluble receptor, IL-6sr) and oncostatin M (OSM) significantly induced alkaline phosphatase (*Alpl*) mRNA expression after 48 hours of treatment, suggesting a selective osteogenic effect. In calvarial osteoblasts treated for 7 days, IL-6 + IL-6sr and OSM stimulated ALPase activity as well as the expression of osteoblastic genes (*Alpl*, *Runx2*, *Sp7*, *Col1a1*) as measured by qPCR. These treatments also enhanced mineralized nodule formation, with OSM already increasing alizarin red staining at 7 days. Both IL-6 + IL-6sr and OSM markedly increased mineralization at 14 days. Western blot analysis demonstrated that IL-6 + IL-6sr and OSM induce phosphorylation of Shc1 and STAT3 in calvarial osteoblasts. Silencing of Shc1 inhibited STAT3 phosphorylation and suppressed *Alpl* expression in response to IL-6 and OSM, confirming that Shc1 is essential for downstream osteogenic signaling. Together, these findings indicate that IL-6 and OSM selectively promote osteoblast differentiation and bone formation by activating the gp130–Shc1–STAT3 axis, thereby reinforcing the direct anabolic potential of specific IL-6 family cytokines in bone.

Keywords: IL-6;osteoblast;gp130.

3127 - Category: MOLECULAR IMMUNOLOGY (MI)

Reducing Aggregation of Single-Chain Antibody Fragments (scFvs) Aiming CAR-T cell therapies

FRANCA, L.V.¹; STUDART, I.C.²; BRIGIDO, M.D.M.¹; MARANHÃO, A.Q.¹. 1. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL; 2. UNIVERSIDADE FEDERAL DO CEARÁ, FORTALEZA - CE - BRASIL.

Multiple myeloma is a type of hematologic cancer of plasma cells that overexpress B-cell maturation antigen (BCMA), and it remains an incurable disease. Immunotherapy with T cells genetically engineered to express chimeric antigen receptors (CAR-T) represents a major progress in the treatment of such malignancies. However, CAR-T cell exhaustion remains a limiting factor in their cytotoxicity and, consequently, in the overall efficacy of this approach. One contributing factor to CAR-T cell exhaustion is the oligomerization of single-chain variable fragments (scFvs) in the extracellular portion of CARs. Therefore, this work aims to design new anti-BCMA scFvs, considering the reduction of their immunogenicity through humanization and, additionally, their aggregation potential, a process herein referred to as harmonization. To this end, a murine anti-BCMA scFv from the literature was the basis for the design of humanized and harmonized versions. The theoretical aggregation potential of the sequences was verified *in silico*, resulting in three scFvs with increasing degrees of predicted aggregation: harmonized, humanized 1, and humanized 2 (or chaotic). The antibodies were expressed in soluble form in *Escherichia coli* strains, after IPTG induction, and purified by chromatographic methods. Electrophoresis-based assays confirmed the antibody expression in the transformed bacteria, with time-dependent accumulation. Size exclusion and dynamic light scattering (DLS) analyses corroborated the predicted aggregation profiles, as the harmonized scFv exhibited lower aggregation compared to the other scFv versions. Furthermore, flow cytometry analyses demonstrated that all scFvs are capable of binding to the BCMA antigen on myeloma cell lines, which is a relevant aspect for their potential application. Thus, these preliminary results contribute to a better understanding of antibody aggregation and suggest that harmonization may be applied to the design of more refined CAR-T cells.

Keywords: scFv;Protein Aggregation;CAR-T Cell Therapy.

3144 - Category: MOLECULAR IMMUNOLOGY (MI)

DEVELOPMENT OF ANTI-CD19 CAR MOLECULES WITH SPACER REGIONS DERIVED FROM HUMAN IgG4

ARAGÃO, D.R.¹; STUDART, I.C.¹; SOUSA, I.G.¹; WERNECK, I.V.T.¹; DE SOUZA, P.V.D.¹; BONAMINO, M.H.²; BRIGIDO, M.D.M.¹; MARANHÃO, A.Q.¹. 1. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL.

Immunotherapies have been established as an effective strategy for treating various types of cancer. Among these approaches, chimeric antigen receptor expressing T-cell therapy (CAR-T) stands out for enabling T lymphocytes to recognize and eliminate cells harboring specific antigens. The structural architecture of the CAR is crucial for its efficacy. Classically target recognition is mediated by the specificity of a scFv (single-chain variable fragment). However, domains such as the spacer region (hinge), which separate the scFv from the CAR transmembrane domain, can also directly impact the activation and functionality of CAR-T cells. This study aims to investigate the impact of different spacer regions derived from human IgG4 on novel anti-CD19 CAR variants. Four distinct spacer region constructs based on the IgG4 hinge and the IgG1 elbow were designed and cloned into the pT4 19BBz vector, which allows transgene delivery via a Sleeping Beauty transposon-based system. The expression of these constructs was evaluated by flow cytometry 24 hours after transfection in the adherent HEK-293T cell line as a preliminary step before functional validation in PBMCs (peripheral blood mononuclear cells). Flow cytometry analysis showed that CAR was expressed in all four constructs, with the H4 and 2HE4 variants exhibiting a higher level of expression (75.5% and 71.8%, respectively), whereas 2H4 and HE4 appeared to express less compared to the other variants (38% and 57.8%, respectively). By investigating how variations in the spacer region of the extracellular portion of the CAR can modulate the performance of CAR-T cells, this study seeks to contribute to the functional understanding of the hinge impact, considering parameters such as flexibility, antigen accessibility, and basal activation (without target) potential, providing support for the development of CARs with optimized performance.

Keywords: Immunotherapy;CAR-T;Hinge Region.

3146 - Category: MOLECULAR IMMUNOLOGY (MI)

Recombinant Human Antibodies for Scorpion Envenomation: Optimizing Serrumab Production Across Heterologous Expression Systems

PIRES, G.M.M.; DE OLIVEIRA, J.L.; PUCCA, M.B.. UNIVERSIDADE ESTADUAL PAULISTA (UNESP), ARARAQUARA - SP - BRASIL.

A seleção de anticorpos monoclonais totalmente humanos via exibição em fagos representa uma estratégia de ponta para o desenvolvimento de antivenenos mais seguros e específicos. O escorpionismo, particularmente o envenenamento por *Tityus serrulatus*, é um grande problema de saúde pública no Brasil, com aproximadamente 200.000 casos relatados anualmente. O tratamento atual depende de soros heterólogos derivados de imunoglobulinas animais, que estão associados a reações adversas e eficácia variável. Nesse contexto, o Serrumab, um anticorpo monoclonal scFv humano direcionado às neurotoxinas de *T. serrulatus*, surge como uma alternativa terapêutica promissora. Este estudo compara dois sistemas de expressão heterólogos, *Escherichia coli* e *Pichia pastoris*, para otimizar a produção de Serrumab recombinante. Em *E. coli*, a expressão foi induzida com IPTG em frascos Erlenmeyer e Tunair, seguido por purificação por afinidade com marcador his. Apesar da ligação confirmada ao veneno via ELISA, a produção foi limitada por baixos rendimentos e contaminação por endotoxina. Para superar essas restrições, o gene scFv foi subclonado no vetor pPICZαA e expresso em *P. pastoris* KM71H. Clones resistentes à zeocina foram selecionados e induzidos com metanol, potencializando a capacidade da levedura de produzir proteínas livres de endotoxina com maiores rendimentos e modificações pós-traducionais. As análises atuais avaliam os níveis de expressão, a pureza e a atividade funcional, utilizando a expressão bacteriana como referência. A avaliação comparativa desses sistemas fornece insights cruciais sobre a escalabilidade e a segurança de plataformas baseadas em leveduras para anticorpos antiveneno recombinantes, impulsionando o desenvolvimento de bioterapêuticos humanos de próxima geração para envenenamento.

Keywords: antivenom;scFv;Pichia pastoris.

3152 - Category: MOLECULAR IMMUNOLOGY (MI)

Exploring the Probiotic Microorganism *Zymomonas mobilis* for the Delivery of Therapeutic Recombinant Antibodies to the Intestinal Mucosa

OLIVEIRA, R.R.¹; RIBEIRO, I.B.D.O.¹; DO ALMO, M.M.¹; DE MORAES, L.M.P.²; MARANHÃO, A.Q.¹; BRIGIDO, M.D.M.¹. 1. ¹MOLECULAR IMMUNOLOGY LABORATORY, DEPARTMENT OF CELL BIOLOGY, DARCY RIBEIRO UNIVERSITY CAMPUS, UNIVERSITY OF BRASÍLIA, BRASÍLIA, FEDERAL DISTRICT - 70910-900, BRAZIL., BRASÍLIA - DF - BRASIL; 2. ²YEAST BIOTECHNOLOGY LABORATORY, CELL BIOLOGY DEPARTMENT, DARCY RIBEIRO UNIVERSITY CAMPUS, UNIVERSITY OF BRASÍLIA, BRASÍLIA, FEDERAL DISTRICT - 70910-900, BRAZIL., BRASÍLIA - DF - BRASIL.

The use of probiotic microorganisms can benefit intestinal health by supporting the maintenance of the gut microbiota. *Zymomonas mobilis*, a bacterium with therapeutic potential, has been used in Brazil to treat intestinal disorders. Here we describe heterologous expressing systems for therapeutic antibodies on the surface of *Z. mobilis*. An anti-CD3 scFv fragment was used to evaluate its efficacy in an ulcerative colitis murine model. However, low expression levels—possibly due to cellular toxicity or the formation of inclusion bodies—limited the therapeutic effect. To overcome this limitation, a tetracycline-repressive expression vector was constructed using the reverse tetracycline repressor (revTetR), with eGFP as a reporter. Additionally, vectors encoding outer membrane anchoring protein InaK fused to the anti-CD3 scFv were developed to compare expression efficiency. *Z. mobilis* were transformed with eGFP expression vector and individual clones were tested for GFP production by spectrofluorimetry. The results confirmed an inverse correlation between eGFP expression and tetracycline concentration, with 83% of cells exhibiting fluorescence. To evaluate scFv expression, *Z. mobilis* with InaK expression vectors was cultured for 24 h with tetracycline (5 µg/mL), followed by 6 h of incubation without the antibiotic. Expression levels were analyzed by flow cytometry. With the use of the InaK anchoring domain, 39% of cells displayed scFv when cultured under tetracycline inhibition, compared to 8% of cells grown without inhibition. This results support the toxic effect of recombinant expression in *Z. mobilis*. In conclusion, the revTetR-regulated system is functional in *Z. mobilis*, representing an innovative approach for recombinant bacteria-based therapies. Improved expression of the anti-CD3 scFv was observed compared to non-regulated vectors, and future studies will investigate the impact of different tetracycline concentrations on antibody production more thoroughly.

Keywords: *Zymomonas mobilis*;anti-CD3; heterologous expression;;reverse tetracycline repressor.

3174 - Category: MOLECULAR IMMUNOLOGY (MI)

Integration of Genomics and Structural Bioinformatics in the Characterization of Genetic Variants Involved in Activated PI3-Kinase Delta Syndrome (APDS)

AGONIGI, B.N.S.¹; CUNHA, D.P.¹; MORGADO, E.S.¹; ZUMA, M.C.C.²; LOPES, M.R.G.¹; BUENO, F.V.D.S.¹; COSTA, M.G.³; FACCION, R.S.¹; GOMES, L.H.F.¹; DE VASCONCELOS, Z.F.M.¹. 1. INSTITUTO FERNANDES FIGUEIRA, RIO DE JANEIRO - RJ - BRASIL; 2. INSITUTO FERNANDES FIGUEIRA, RIO DE JANEIRO - RJ - BRASIL; 3. PROGRAMA DE COMPUTAÇÃO CIENTIFICA, RIO DE JANEIRO - RJ - BRASIL.

Activated PI3-Kinase Delta Syndrome (APDS) is a rare, autosomal dominant inborn error of immunity (IEI) caused by pathogenic variants in the *PIK3CD* or *PIK3R1* genes. Clinically, APDS presents a broad phenotypic spectrum, most notably recurrent sinopulmonary infections, lymphoproliferative disorders, chronic enteropathy, and autoimmune features. Due to its clinical overlap with other primary immunodeficiencies, diagnosis is frequently delayed, with an average lag of up to seven years. This study seeks to identify and functionally characterize genetic variants associated with APDS from both genomic and structural standpoints in individuals with clinical suspicion of IEI. Participants were recruited through the Brazilian National Network for Genomics of Inborn Errors of Immunity (RENOMIEII). Whole genome sequencing (WGS) of family trios is being performed, integrating clinical, laboratory, and genomic data. The analysis targets missense variants, indels, and copy number variants (CNVs) in *PIK3CD* and *PIK3R1*, applying ACMG guidelines for clinical interpretation. Variant assessment incorporates state-of-the-art computational tools, including AlphaMissense for point mutations and ISV/AnnotSV for CNVs, with structural data and population frequencies also considered. Structural modeling of both wild-type and mutant proteins is conducted using AlphaFold 3.0, followed by molecular dynamics simulations via NAMD software. These analyses aim to elucidate how genetic alterations may disrupt critical domains and functional binding sites of PI3K δ complex components. To date, 52 patients have been sequenced by the RENOMIEII project (27 females and 25 males). Key phenotypic descriptors based on Human Phenotype Ontology (HPO) include fever (7.7%), abscess (7.7%), splenomegaly (5.8%), recurrent viral infections (5.8%), pneumonia (3.8%), elevated IgE (3.8%), and hypogammaglobulinemia (3.8%). Findings may clarify APDS, enrich variant databases, and support structural genomic analysis.

Keywords: Inborn Errors of Immunity;Genetic Diagnosis;Molecular Dynamics.

3190 - Category: MOLECULAR IMMUNOLOGY (MI)

Delivery of Monoclonal Antibodies for Flavivirus Therapy using DNA and mRNA vectors

RODRIGUES, L.S.; BARBOSA, A.C.A.; PINHEIRO, M.C.M.; FRANÇA, R.K.A.D.O.; MARANHÃO, A.Q.; BRIGIDO, M.D.M.. UNB, BRASÍLIA - DF - BRASIL.

Zika virus (ZIKV) and dengue virus (DENV) are important arboviral diseases in Brazil, both caused by members of the Flaviviridae family. Although a dengue vaccine was recently approved, no effective treatment or vaccine is currently available for ZIKV. Monoclonal antibodies (mAbs) have shown promise in antiviral therapies, but their application against flaviviruses is limited not only by the risk of antibody-dependent enhancement (ADE), often associated with the antibody Fc region, but also by the high cost and complexity of large-scale recombinant protein production. To address these challenges, we engineered recombinant mAbs in an FvFc format, fusing single-chain variable fragments (scFvs) to the Fc domain of human IgG2 and the extended hinge region of IgG3. This design aims to enhance neutralizing potential while minimizing ADE. As a cost-effective alternative to protein production in mammalian cells, we explored gene-based delivery of mAbs using mRNA encapsulated in lipid nanoparticles (LNPs) and DNA vectors. Genes encoding different antibody formats were cloned into expression vectors and transfected into Expi293 cells. Transfection efficiency was monitored via GFP expression, and antibodies were purified using Protein A affinity chromatography. Binding to ZIKV and the four DENV serotypes was evaluated by ELISA. Both IgG2 and IgG/3 FvFc-formatted mAbs recognized ZIKV and the four DENV serotypes. The IgG/3 FvFc construct demonstrated neutralizing activity in plaque reduction neutralization tests (PRNT) against ZIKV, DENV-1, and DENV-2. mRNA encoding the optimized antibody was encapsulated in LNPs and used to transfect Expi293 cells, with expression confirmed by Western blot. Next, in vivo administration in mice will assess expression, stability, and protective efficacy against ZIKV infection. These findings support the potential of in vivo expression of mAbs by mRNA or DNA delivery strategies as innovative therapeutic approaches for emerging flaviviruses.

Keywords: Lipid nanoparticle;Monoclonal Antibodies;Flavivirus.

3282 - Category: MOLECULAR IMMUNOLOGY (MI)

DEVELOPMENT AND EVALUATION OF A RECOMBINANT NANOVACCINE WITH THE rPKNG PROTEIN FROM CORYNEBACTERIUM PSEUDOTUBERCULOSIS

DANTAS, F.B.; DOS SANTOS, R.M.; RIBEIRO, M.B.; SILVA, M.M.P.E.; VELLOSO, V.C.B.; DOS SANTOS, B.G.D.F.; MEYER, R.; ASSIS, M.S.; BORGES JUNIOR, E.V.; ALCÂNTARA, D.A.D.S.; SANTOS, S.D.S.; MARCHIORO, S.B..
PROGRAMA DE PÓS GRADUAÇÃO EM IMUNOLOGIA, SALVADOR - BA - BRASIL.

Caseous lymphadenitis (CLA) is a chronic disease caused by *Corynebacterium pseudotuberculosis*, leading to significant economic losses in sheep and goat farming. The limited efficacy of available commercial vaccines to combat the disease motivates the development of new strategies. This study aimed to develop a nanovaccine containing the recombinant rPknG protein encapsulated in polymeric nanoparticles, evaluating its physicochemical properties and immunoprotective performance in a murine model. The rPknG protein was obtained by heterologous expression in *Escherichia coli* BL21(DE3) and purified by affinity chromatography, with previously validated identity. The final protein concentration was determined to be 0.80 mg/mL using the Lowry method. Encapsulation was performed by nanoemulsion using polycaprolactone. Physicochemical characterization by *Dynamic Light Scattering* showed average particle sizes of 180 ± 5.9 nm (with rPknG) and 157.5 ± 5.1 nm (without rPknG), PDI < 0.2, and zeta potentials ranging from -16.21 mV to -26.03 mV, indicating stability and homogeneity of the formulations. BALB/c mice were immunized subcutaneously with two doses 21 days apart and divided into six groups: G1 (0.9% saline solution), G2 (encapsulated rPknG), G3 (rPknG + adjuvant), G4 (rPknG), G5 (nanoparticles without antigen), and G6 (adjuvant). After intraperitoneal challenge with the virulent *C. pseudotuberculosis* MIC6 strain, group G2 showed a survival rate of 43% at the end of 30 days, followed by G3 (50%) and G4 (70%), while the remaining groups showed no protection. Statistical analysis revealed a significant difference among the groups ($p = 0.0006$), reinforcing the protein's role as a way to combat CLA. Complementary immunological (antibodies and cytokines), molecular, and histological analyses are underway to understand the underlying mechanisms better and optimize future vaccine formulations.

Keywords: nanovaccine;rPknG;Corynebacterium pseudotuberculosis.

3337 - Category: MOLECULAR IMMUNOLOGY (MI)

INVESTIGATING STING–NLRP3 CROSSTALK IN AUTOPHAGY REGULATION

DE ALMEIDA, V.M.¹; MATTEUCCI, K.C.²; COUILLIN, I.³; ALVES-FILHO, J.C.²; RITEAU, N.⁴. 1. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL; 2. RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. INEM, CNRS, ORLEANS - FRANÇA; 4. INEM-CNRS AND RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO, ORLEANS - SP - BRASIL.

Autophagy is a critical cellular process that maintains homeostasis and modulates inflammatory responses. Recent studies identified the Stimulator of Interferon Genes (STING), a cytosolic DNA sensor, as a key regulator of autophagy. However, the precise molecular mechanisms remain unclear. This preliminary study aims to investigate how the STING–NLRP3 crosstalk regulates autophagy and to uncover the molecular mechanisms underlying this interaction. Preliminary data demonstrated that the STING agonists cGAMP and DiABZI differentially induce IRF3 and TBK1 phosphorylation, peaking at 30 minutes and 3 hours, respectively. Both agonists enhanced LC3-II protein expression and LC3 puncta formation in wild-type Bone-marrow-derived macrophages (BMDMs). These effects were abolished in STING-deficient macrophages, underscoring the essential role of the STING pathway in autophagy regulation. Confocal microscopy further revealed significant colocalization of LC3 and the lysosomal marker LAMP-1, especially upon DiABZI stimulation, indicating functional autolysosome formation dependent on STING. Autophagic flux analysis, using lysosomal inhibitor bafilomycin, further demonstrated that STING signaling is required for effective autophagy progression. Notably, preliminary experiments also indicated impaired autophagic flux in NLRP3-deficient BMDMs upon STING agonist treatment, suggesting a previously unexplored functional interplay between STING and NLRP3 pathways in autophagy regulation. Collectively, these preliminary findings support the hypothesis that the STING-NLRP3 axis plays an important role in regulating autophagy in macrophages. Ongoing studies aim to dissect the underlying molecular interactions and assess the potential of targeting this pathway in chronic inflammatory settings, such as pulmonary fibrosis.

Keywords: STING;NLRP3;Autophagy.

3359 - Category: MOLECULAR IMMUNOLOGY (MI)

Transcriptome analysis of circulating microRNAs associated with Chagas disease susceptibility and its regulation of immune-related genes

DE ARAUJO, F.M.D.S.¹; ROMA, E.H.¹; RENZETTI, A.R.D.S.¹; PORTELA, L.F.¹; DA COSTA, F.P.¹; HASSLOCHER-MORENO, A.M.¹; DE HOLANDA, M.T.¹; SANGENIS, L.H.C.¹; DA COSTA, A.R.¹; LANNES-VIEIRA, J.²; DE ARAÚJO-JORGE, T.C.²; FERREIRA, R.R.²; SARAIVA, R.M.¹; BONECINI, G.¹. 1. INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO OSWALDO CRUZ/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Chagas disease (CD) is a major public health concern in Latin America. Understanding the molecular mechanisms driving disease progression and identifying biomarkers are crucial. This study investigates the role of microRNAs (miRNAs) in CD susceptibility and Chronic Chagas Cardiomyopathy (CCC) progression. A prospective observational study was conducted with CD patients and healthy controls (HC). Sequencing of circulating miRNAs, differential expression and functional analysis was performed (Ingenuity Pathway Analysis software), focusing on immune response pathways (IR-P). We identified 40 differentially expressed miRNAs between CD group and HC. Of those, we highlight the upregulation of miR199a-5p in the CD group, predicting downregulation in the expression of 51 IR-P genes, contributing in the downregulation of proliferation, differentiation and development of lymphocytes. The overexpression of miR-153-3p and -143-3p in CD group may be related to downregulation of 33 and 48 IR-P target genes interacting with decreasing lymphocytes proliferation, differentiation, and cell numbers, influencing negatively in the activation of B and T lymphocytes as well as decreasing in the development of Th1 cells. Upregulation in miR-1228-5p in severe compared to moderate CCC is predicted to downregulate 20 IR-P genes, potentially impairing leukocyte migration and myeloid cell recruitment. Conversely, the downregulation of miR-27b-5p, promotes the upregulation of 16 IR-P, enhancing lymphocyte activation, leukocyte viability, and T lymphocyte cell cycle progression. On the other hand, the upregulation of miRNA-1285-3p in severe versus mild CCC, predict downregulate 43 IR-P genes influencing negatively the proliferation of T lymphocytes, differentiation of mononuclear leukocytes and in the number of T and Th17 cells. This study provides evidence for the dysregulation of specific miRNAs in CD, highlighting their potential as biomarkers for monitoring disease infection and progression.

Keywords: miRNA;Chagas disease;Immune-related genes.

3371 - Category: MOLECULAR IMMUNOLOGY (MI)

X-linked hyper-IgM syndrome with CD40LG mutation: two case reports Brazilian patients

BUENO, F.V.D.S.¹; MATIAS, D.O.²; AGONIGI, B.N.D.S.¹; CUNHA, D.P.¹; DE MELO, M.A.F.¹; DOS SANTOS, C.R.¹; COSTA, M.G.³; GOUDOURIS, E.S.⁴; MARINHO, A.K.B.B.⁵; PEREIRA, G.M.B.²; FACCION, R.S.¹; DE VASCONCELOS, Z.F.M.¹. 1. INSTITUTO NACIONAL DE SAÚDE DA MULHER, DA CRIANÇA E DO ADOLESCENTE FERNANDES FIGUEIRA (IFF/FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 2. OSWALDO CRUZ INSTITUTE (IOC), RIO DE JANEIRO - RJ - BRASIL; 3. FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 4. INSTITUTO DE PUERICULTURA E PEDIATRIA MARTAGÃO GESTEIRA (IPPMG), RIO DE JANEIRO - RJ - BRASIL; 5. HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Introduction: X-linked hyper-IgM syndrome (XHIGM) is a rare inborn error of immunity characterized by low serum IgG, IgA, and IgE with normal or elevated IgM, caused by mutations in CD40 ligand (CD40L) impairing immunoglobulin class-switch recombination. Here we report two patients with CD40L variants in exon 5 affecting the TNF domain. Methods: DNA was extracted from peripheral blood and/or oral swabs for panel and whole-exome (WES), and Sanger sequencing. Peripheral blood mononuclear cells were isolated, then stimulated or left unstimulated for 7 hours at 37°C. CD40L expression was assessed on control and activated CD4+ T cells by flow cytometry (FC), and structural modeling of CD40L was performed. Results: The first case was a 1-year-old boy, initially diagnosed with common variable immunodeficiency, presenting with severe respiratory infection with *Pneumocystis jirovecii*. Serology showed low IgG and IgA, and normal IgM. Genetic analysis revealed a hemizygous CD40L missense variant of uncertain significance (c.515A>G; p.Tyr172Cys), with parental segregation further supporting this variant as candidate. FC indicated absence of CD40L surface expression on activated CD4+ T cells, suggesting disrupted immune signaling. The second case was a 3-year-old boy with recurrent viral pneumonia, neutropenia, and post-vaccine measles showed low IgG and IgA, and high IgM. WES identified a likely pathogenic frameshift variant (c.492_493del; p.Arg165Thrfs*35), parental segregation is yet to be confirmed. FC revealed reduced CD40L surface expression on activated CD4+ T cells compared to controls. Structural modeling suggested the missense variant destabilizes the protein, while the frameshift likely results in truncation, both impairing CD40/CD40L interaction. Conclusion: These findings highlight phenotypic and molecular variability in XHIGM and demonstrate the importance of integrating immunophenotyping and molecular approaches to improve variant interpretation and diagnosis.

Keywords: CD40 ligand;x-linked hyper IgM syndrome;variant.

3389 - Category: MOLECULAR IMMUNOLOGY (MI)

Genetic Engineering of *Saccharomyces boulardii* as a Recombinant Platform for Surface-Displayed Antibody Production

PINHATE, S.B.; DE MATOS, A.L.T.; GUIMARÃES, P.O.; MARANHÃO, A.Q.; BRIGIDO, M.D.M.. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

The yeast *Saccharomyces boulardii* was evaluated as a biotherapy platform for recombinant antibody production. Our goal is to achieve the display of a therapeutic antibody scFv of anti-CD3, anchored to a protein extender, AGA, to improve the fragment's display on the cellular wall. To evaluate the impact of the heterologous production of the scFv fragment of the antibody anti-CD3 with the proteic extensor, were performed the following experiments: Growth curve, in which the cultures (Wild Type - WT, and Recombinant type) were monitored between 0 to 48 hours in YPD medium added with 200ug/ml of geneticin, measuring the OD regularly, we could see that the recombinant protein production did not affect the cell growth, neither its viability; Confocal microscopy, where cells were labeled to evaluate the production of the antibody anti-CD3 with anti Flag followed by anti Rabbit IgG - FITC along with Propidium iodide (PI) to assess viability, confirmed the maintenance of cell morphology without any qualitative differences in format or size of the recombinant cells comparing to the WT; Flow cytometry, prepared as for the microscopy samples, demonstrated that more than 90% cells in both types were kept viable. Since the SED promoter used in the expression vector is activate under stress conditions, we evaluate if we can improve cell production growing the recombinant yeast under stress (NaCl 0.5M and ethanol 20%) for 15 minutes and analysis by flow cytometry. We observed an increase of 3 times in scFv production, but also a significant impact in cell viability. These results show that the heterologous production of the scFv anti-CD3 anchored to AGA extensor didn't compromise the yeast metabolism, corroborating *S. boulardii* to be an efficient system for biotechnological applications.

Keywords: Heterologous production; *Saccharomyces boulardii*; Antibody display.

3394 - Category: MOLECULAR IMMUNOLOGY (MI)

THE ROLE OF EZH2 IN CD8 T CELL DIFFERENTIATION AND ANTITUMOR RESPONSE

PASSOS, T.D.O.; DA SILVA, G.B.L.; MELO, G.A.; CRUZ, C.C.O.D.M.; NETO, M.A.N.; DE OLIVEIRA, J.P.D.L.; CRUZ, P.C.O.D.M.; WERNECK, M.B.D.F.; PEREIRA, R.D.M.S.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - UFRJ, RIO DE JANEIRO - RJ - BRASIL.

In chronic infections and cancer, CD8 T cells undergo a progressive decrease of effector function, loss of proliferation capacity and gain of inhibitory receptors. This loss of functionality, known as T cell exhaustion, also affects CAR (Chimeric Antigen Receptor) T cells immunotherapy for solid tumors. Our prior data in a viral exhaustion model, the murine LCMV clone 13 infection, revealed that early generated exhausted CD8 T cells expressed lower levels of the epigenetic modulator Ezh2 (Enhancer of zeste homolog 2) which is the catalytic subunit of the Polycomb Repressor Complex 2, that trimethylates histone 3 at the lysine 27, and has been reported as critical to CD8 T cell effector differentiation and antitumor response. Here, we seek to evaluate the role of Ezh2 in CD8 T cell differentiation and the relevance of modulating its levels for advance in experimental immunotherapies. In vitro activated Ezh2 deficient CD8 T cells from Ezh2^{fl/fl} Cd4 Cre mice exhibited impaired proliferation and production of IFN- γ and TNF, along with increased expression of exhaustion associated molecules PD-1, TIM-3 and CD38. In vivo, Ezh2 overexpression in CD8 CAR T cells targeting mice B16-hCD19 melanoma led to an increase in Ki67 and granzyme B production, but did not provide significant gain in restraining tumor size when compared to control groups. Thus, further investigations on how and when Ezh2 is required for proper CD8 T cell function are necessary. In a preliminary experiment aiming to clarify the time-related effects of Ezh2 in CD8 T cells, early pharmacological inhibition led to the exhaustion phenotype and loss of proliferation while inhibition in a later time point during differentiation increased memory-related molecules and preserved Ki67 expression (CEUAs: 041/20 and 107/23). Together, our results suggest the coupling of proliferation and differentiation under Ezh2 control during the activation of CD8 T cells and afterwards Ezh2 restriction of a stemness profile.

Keywords: CD8 T lymphocytes; Ezh2; CAR T cells.

3413 - Category: MOLECULAR IMMUNOLOGY (MI)

One-step generation of TCR knock-in mice targeted to the TCR β locus results in functional mature T Cells

BORTOLATTO, J.¹; BILANOVIC, J.¹; DUAN, S.²; SCHMIDT, F.³; HARTWEGER, H.⁴; BJØRNSDOTT, V.⁵; ESCOLANO, A.⁶; THOMAS, P.G.⁷; VICTORA, G.¹; BILATE, A.M.⁸; JACOBSEN, J.T.⁹. 1. THE LABORATORY OF LYMPHOCYTE DYNAMICS, THE ROCKEFELLER UNIVERSITY, NY - ESTADOS UNIDOS DA AMERICA; 2. ST. JUDE CHILDREN'S RESEARCH HOSPITAL, DEPARTMENT OF IMMUNOLOGY, TN - ESTADOS UNIDOS DA AMERICA; 3. LABORATORY OF RETROVIROLOGY, THE ROCKEFELLER UNIVERSITY, NY - ESTADOS UNIDOS DA AMERICA; 4. LABORATORY OF MOLECULAR IMMUNOLOGY, THE ROCKEFELLER UNIVERSITY, NY - ESTADOS UNIDOS DA AMERICA; 5. THE UNIVERSITY OF OSLO, DEPARTMENT OF CLINICAL MEDICINE, OSLO - NORUEGA; 6. LABORATORY OF IMMUNOBIOLOGY AND VACCINES, VACCINE AND IMMUNOTHERAPY CENTER, THE WISTAR INSTITUTE, PA - ESTADOS UNIDOS DA AMERICA; 7. ST. JUDE CHILDREN'S RESEARCH HOSPITAL, DEPARTMENT OF IMMUNOLOGY, NY - ESTADOS UNIDOS DA AMERICA; 8. THE LABORATORY OF MUCOSAL IMMUNOLOGY, THE ROCKEFELLER UNIVERSITY, NY - ESTADOS UNIDOS DA AMERICA; 9. 7. THE UNIVERSITY OF OSLO, DEPARTMENT OF CLINICAL MEDICINE, OSLO - NORUEGA.

Transgenic mouse models expressing predefined T cell receptors (TCRs) have been instrumental in advancing our understanding of T cell biology. However, these models rely on random genomic insertion of large constructs, require labor-intensive embryo manipulation, and frequently result in aberrant TCR expression and phenotypes. These limitations render TCR transgenic models insufficient to meet the mounting demands for rapid and precise model systems to evaluate TCR specificities. To address these challenges, we developed a streamlined method that combines Adeno-Associated Virus (AAV), coupled with CRISPR/Cas9 genome editing to precisely integrate pre-rearranged TCR α /b sequences into the mouse *Trb* locus, enabling the rapid generation of TCR knock-in mice with physiological TCR expression and functional T cell development and differentiation. Our approach enables the rapid generation of TCR monoclonal models through a universally optimized AAV vector system, significantly enhancing the versatility and utility of monoclonal TCR mice in basic immunology and preclinical research such as cancer immunotherapy and vaccine development.

Keywords: Adeno-Associated Virus (AAV); T cell receptors (TCRs); CRISPR/Cas9 genome editing.

3448 - Category: MOLECULAR IMMUNOLOGY (MI)

EVALUATION OF OXIDATIVE STRESS IN SERUM OF PATIENTS WITH RHEUMATOID ARTHRITIS IN NORTHERN MATO GROSSO

RODRIGUES, A.J.R.¹; NETO, F.P.D.S.¹; BARBOZA, F.²; SINHORIN, V.D.G.³; ALBIERO, L.R.⁴. 1. FACULDADE ATENAS, SORRISO - MT - BRASIL; 2. CLINICA CIMED, SINOP - MT - BRASIL; 3. UNIVERSIDADE FEDERAL DO MATO GROSSO, CUIABÁ - MT - BRASIL; 4. FACULDADE ATENAS, SINOP - MT - BRASIL.

Introduction: Rheumatoid Arthritis (RA) is a systemic autoimmune disease, being one of the most prevalent among rheumatic diseases. Oxidative stress is generated from the imbalance between oxidant and antioxidant compounds in the body, resulting in cellular and tissue damage. **Objective:** The present study sought to evaluate in vitro the oxidative stress of patients with RA in northern Mato Grosso. **Methodology:** The following parameters were evaluated: reduced glutathione (GSH), ascorbic acid (ASA), carbonyl proteins (Carbonyl, protein damage) and Thiobarbituric Acid Reactive Substances (TBARS, lipid damage). The experimental group consisted of patients diagnosed with RA, in the active phase, who used exclusively Methotrexate and Prednisone (5-20 mg) and the control group, consisting of healthy individuals. The sample groups were equivalent in age, sex, immunogenic profile and non-use of medications, in addition to those established for the experimental group. For statistical analysis, the t-test or Mann-Whitney test was performed and correlations were performed for those that showed a significance level established at $P < 0.05$. **Results:** The activities of the non-enzymatic antioxidants GSH and ASA, as well as the oxidative stress markers TBARS and Carbonyl, did not show any significant difference when comparing the AR group with the control group. **Conclusion:** In this study, oxidative stress parameters in the serum of rheumatoid arthritis patients from northern Mato Grosso were analyzed and compared with those of healthy individuals. The activities of the biomarkers analyzed showed no statistically significant differences, which may suggest that these response patterns are regional specificities.

Keywords: Rheumatoid arthritis; Oxidative stress; Autoimmunity.

3479 - Category: MOLECULAR IMMUNOLOGY (MI)

Regulation of HERVs by Sars-Cov-2 and the potential association with the persistence of coagulopathy and thromboinflammation in post-acute COVID-19 syndrome (PACS).

ROZINI, S.V.¹; GONCALVES, R.M.¹; FINTELMAN-RODRIGUES, N.²; DE PAULA, A.³; SOUZA, T.M.L.³; BOZZA, P.T.³.
1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. FIOCRUZ-RJ, RIO DE JANEIRO - RJ - BRASIL.

Human endogenous retroviruses (HERVs), which constitutes about 8% of the human genome, can be reactivated and have been associated with various pathologies such as cancer and autoimmune diseases. During SARS-CoV-2 infection, HERV-K reactivation was observed in severe COVID-19 cases and correlated with increased thromboinflammatory markers. Patients with post-acute COVID-19 syndrome (PACS) also showed elevated levels of D-dimer, inflammatory cytokines, and HERV-W proteins in plasma. This study aimed to investigate the mechanisms of HERV regulation by SARS-CoV-2 and its association with thromboinflammation in PACS. Survivors of moderate or severe COVID-19 were enrolled 30 days to 36 months post-discharge (CAAE 5523520.3.0000.5249). Persistent symptoms were recorded via questionnaire, and plasma was isolated from citrate-treated whole blood of PACS patients or healthy controls. After centrifugation, platelet-poor plasma was stored at -80 °C. RNA sequencing (RNA-seq) was performed using the MGISEQ-2000 platform and analyzed with Galaxy. Inflammatory cytokines were quantified by ELISA. Plasma samples from 62 PACS patients (31–194 days, PCO1) and 23 patients (2–3 years, PCO2) were analyzed. Increased levels of cytokines such as Tissue Factor, soluble P-selectin (sCD62p), ANG1, and PDGF were observed in both PACS groups compared to controls. Notably, sCD62p and ANG1 levels were lower in PCO2 compared to PCO1. RNA-seq analysis revealed differential expression of HERV-related genes, with six HERV elements consistently upregulated in both PACS groups. A positive correlation was found between P-selectin/ANG1 levels and expression of these HERVs, suggesting a persistent HERV upregulation associated with thromboinflammation. These findings support a sustained genetic reactivation of HERV-derived elements in PACS patients, potentially contributing to chronic inflammatory processes and the pathophysiology of long COVID.

Keywords: Human endogenous retroviruses;long-covid;thromboinflammation.

2725 - Category: NEUROIMMUNOLOGY (NI)

EVALUATION OF THE IMPACT OF ACADEMIC STRESS ON THE PRODUCTION OF PRO-INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES IN PSYCHOLOGY AND MEDICAL STUDENTS IN THE DEPARTMENT OF CAUCA DURING AN ACADEMIC SEMESTER

LUNA, N.; CUELLAR, R.A.D.. UNIVERSIDAD DEL CAUCA, POPAYÁN - COLOMBIA.

Academic stress is a significant issue that affects individuals due to various stressors, including academic overload, assignment completion, exam preparation, and familial, social, and economic difficulties. This combination of factors can negatively impact emotional health and lead to dysregulation of the immune system, potentially triggering inflammatory processes. This study aimed to evaluate the impact of academic stress on the production of pro-inflammatory and anti-inflammatory cytokines in psychology and medical students from the Department of Cauca during an academic semester. To achieve this, a longitudinal descriptive study of a quantitative nature was conducted, involving 112 students enrolled in the fourth and fifth semesters of the psychology and medicine programs at Fundación Universitaria de Popayán (University Foundation of Popayán) and Universidad del Cauca (University of Cauca), respectively. Perceived stress was assessed using the SISCO Inventory. Sociodemographic conditions were characterized through a survey, and the expression of cytokines IL-6, TNF- α , and IL-10 was analyzed using the ELISA (Enzyme-Linked Immunosorbent Assay) technique at four time points throughout the semester. The results indicated that the perceived stress level remained consistently moderate throughout the semester for both medical and psychology students. The most prevalent stressors identified were: "work overload," "limited time to complete assignments," and "examinations." The symptoms reported most frequently and intensely by both groups included: "drowsiness," "anxiety," and "difficulty concentrating." Medical students displayed a sustained inflammatory profile (a progressive increase in IL-6) combined with an early anti-inflammatory response (IL-10), followed by a subsequent decline. In contrast, psychology students exhibited a gradual increase in IL-6 and a delayed but significant anti-inflammatory response (IL-10).

Keywords: Academic stress;Cytokines;Psychology and Medical students.

2795 - Category: NEUROIMMUNOLOGY (NI)

NEUROBEHAVIORAL AND HEPATIC ALTERATIONS IN AN IN VIVO MODEL OF HTLV-1 INFECTION: IMPLICATIONS FOR THE PATHOGENESIS OF HAM/TSP.

MONTEIRO, G.M.; CAMPOS, M.C.S.; PIMENTA, J.V.; BERBERT, L.; CONSTANCIO, C.D.S.; COBOS, E.M.; NAZIOBERTO DUDA DE FARIAS, J.; LIMA, J.E.N.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Human T Lymphotropic Virus Type-1-Associated Myelopathy (HTLV-1 (HAM)) is a chronic and progressive disease. The spastic paraparesis, cognitive/autonomic dysfunctions, often with a strong central nervous system inflammation, characterize this disease. In addition, HTLV-1 infection appears to promote systemic homeostatic imbalance. Moghadam *et al.* (2018) showed that BALB/c mice infected with HTLV-1-infected MT-2 cells (i.p.) develop HAM-like symptoms within 2 months. This study aimed to validate the murine model of HTLV-1 infection and investigate hepatic alteration (CEUA 080/24). After 60 days, motor, cognitive, and behavioral tests were performed in two groups (n = 5) of female BALB/c mice (4 weeks old): control group (PBS) and infected group (10⁶ MT-2 cells). HTLV-1 infection did not affect body weight, but behavioral changes were observed. In the open field test, the locomotor parameters are equal, but the infected mice showed more time in the corners (anxiety-like behavior). Footprint tests showed forelimb impairment; the hanging wire test indicated reduced muscle strength and motor neuron integrity. In the pole test, mice showed delayed movement, suggesting motor impairment. Infected mice also showed increased immobility time in the tail suspension test (depressive-like behavior). To analyze if HTLV-1 infection alters liver structure and physiology, animals were euthanized and organs collected. The liver was fixed in 10% buffered formalin for 72h, paraffin-embedded, sectioned, and stained with Hematoxylin and Eosin (H&E), Picrosirius Red (PS) for collagen, and specific antibodies to evaluate T lymphocyte infiltration by immunofluorescence. The histological analysis revealed no collagen alterations (PS staining) nor hyperemia (H&E). However, infected animals presented an inflammatory infiltrate characterized by T cells. These motor and hepatic inflammatory changes may relate to disease development, although further studies are needed to clarify the mechanisms.

Keywords: HTLV-1;HAM;Liver .

2832 - Category: NEUROIMMUNOLOGY (NI)

Leptin Receptor Signaling in Microglia Modulates Hypothalamic Inflammation and Peripheral Metabolic Dysfunction in Obesity

SANTOS, W.G.S.; CASTRO, G.; ANGELIM, M.K.S.C.; DA SILVA, G.R.; DOS REIS, L.M.; MARTINS, A.J.E.; DOS SANTOS, T.P.; VIEIRA, P.M.M.D.M.. UNICAMP, CAMPINAS - SP - BRASIL.

Obesity is associated with hypothalamic inflammation, which is driven by elevated levels of leptin and free fatty acids in the central nervous system (CNS). Microglia are key sensors of these metabolic changes, yet the specific contribution of leptin receptor (ObR) signaling in microglial activation remains unclear. Here, we investigated how leptin signaling in microglia contributes to inflammation and systemic metabolic alterations during high-fat diet (HFD)-induced obesity. ObR^{flxed} mice were crossed with CX3CR1^{CreER}:R26^{DsRed} mice. These mice were treated with tamoxifen on day 3 post-birth to generate animals with microglia-specific deletion of ObR (ObR KO). After 20 weeks on HFD, ObR deletion did not affect body weight or food intake. However, HFD-fed ObR KO mice showed a higher frequency of TNF⁺ microglia in the hypothalamus. In the periphery, both ObR KO and control-fed mice developed insulin resistance on an HFD, with ObR KO mice exhibiting greater insulin sensitivity. Moreover, HFD-fed ObR KO mice displayed a reduced number of pancreatic islets and were protected from HFD-induced hepatic steatosis, with no significant changes in adipocyte size or epididymal white adipose tissue (eWAT) mass. In vitro, primary microglia treated with leptin (100 ng/mL) and palmitate (400 μ M) for 6 hours showed increased mitochondrial surface area, volume, and branching, alongside enhanced glycolytic capacity, without changes in mitochondrial respiration. This treatment also elevated IL-6 expression, suggesting increased microglial reactivity to lipid stimuli. Notably, ObR deletion abolished these effects, indicating that leptin's action on microglia is ObR-dependent. Collectively, our findings demonstrate that leptin receptor signaling in microglia plays a pivotal role in regulating hypothalamic inflammation and direct impact on peripheral metabolic organs such as the liver and pancreas.

Keywords: Mitochondrial dynamics;Neuroimmunometabolism;Palmitate.

2847 - Category: NEUROIMMUNOLOGY (NI)

PI3K γ Deletion Potentiates Cannabidiol's Anti-Inflammatory Effects in Experimental Autoimmune Encephalomyelitis

DA SILVA, L.C.¹; LIMA, I.V.D.A.¹; MOREIRA, F.D.A.²; TEIXEIRA, M.M.²; DE OLIVEIRA, A.C.P.²; FERREIRA, A.P.¹. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Multiple sclerosis (MS) is a chronic, immune-mediated, demyelinating disease of the central nervous system (CNS), affecting over 2.8 million people worldwide. It is characterized by autoreactive lymphocyte infiltration, glial activation, and progressive neurodegeneration, often resulting in motor, sensory, and cognitive impairments. Current therapies are only partially effective, with limited impact on long-term disability. Thus, identifying novel molecular targets and immunomodulatory agents remains critical. Among emerging candidates, cannabidiol (CBD)—a non-psychotomimetic cannabinoid—has shown promise due to its anti-inflammatory, neuroprotective, and immunoregulatory properties. Parallely, the PI3K/Akt/mTOR signaling pathway plays a central role in immune cell activation, migration, and survival, and its dysregulation has been implicated in MS pathogenesis. In this study, we used the experimental autoimmune encephalomyelitis (EAE) animal model to investigate whether genetic deletion of PI3K γ modulates neuroinflammation and enhances the efficacy of CBD. Female C57BL/6 wild-type (WT) and PI3K γ knockout (KO) mice (6-8 weeks old) were immunized with MOG35–55 peptide and treated with CBD or vehicle for 10 days starting at symptom onset. KO-EAE mice exhibited delayed disease onset, lower clinical scores, reduced CNS inflammation and demyelination, and decreased the levels of proinflammatory cytokines (IFN- γ , IL-17, IL-6) and chemokines (CCL5, CCL20) in the spinal cord and inguinal lymph nodes. Glial density (Iba-1+ and GFAP+ cells) and TUNEL+ cells were also attenuated. CBD treatment further potentiated these effects, particularly in KO animals. These findings indicate a synergistic interaction between PI3K γ deletion and CBD treatment, supporting combined therapeutic strategies targeting the PI3K γ /Akt/mTOR axis and the endocannabinoid system in EAE and MS.

Keywords: MS/EAE;PI3K γ ;CBD.

2852 - Category: NEUROIMMUNOLOGY (NI)

Role of hydroxycarboxylic acid receptor 2 (HCAR2) in the development of neuropathic pain

DE CARVALHO, B.O.M.; MENDES, A.S.; DE ANDRADE, L.P.; GOMES, F.I.F.; CUNHA, F.Q.; ALVES FILHO, J.C.F.; CUNHA, T.M.. UNIVERSIDADE DE SÃO PAULO (USP), RIBEIRÃO PRETO - SP - BRASIL.

Neuropathic pain is a chronic condition caused by a lesion or disease of the somatosensory system. Current treatments offer limited efficacy and are associated with adverse effects, underscoring the need for novel therapeutic targets. The hydroxycarboxylic acid receptor 2 (HCAR2) is a G protein-coupled receptor first identified in adipocytes as an inhibitor of lipolysis. Subsequent studies revealed that HCAR2 is expressed in several immune cell types, and its activation modulates neuroinflammation in diverse neurological diseases, including Alzheimer's disease and multiple sclerosis. Given the involvement of neuroinflammation in abnormal neuronal activation under pain conditions, we aim to investigate the role of HCAR2 in the development of neuropathic pain. We observed that pharmacological activation of HCAR2 by intrathecal administration of sodium butyrate reduced mechanical allodynia in wild-type mice 7 days after induction of Spared Nerve Injury (SNI) model. Moreover, global deletion of *Hcar2* did not alter basal nociceptive behavior; however, after SNI, *Hcar2* knockout mice exhibited increased mechanical allodynia compared to wild-type controls. Immunofluorescence analysis revealed predominant HCAR2 expression in spinal cord microglia. 10 days after SNI, stronger and more clustered staining was observed in the ipsilateral dorsal horn compared to naive mice, suggesting that nerve injury increases HCAR2 expression. To further investigate the role of HCAR2 in microglia, we performed a primary culture, stimulated cells with LPS (10 ng/mL), and treated them with HCAR2 agonists (nicotinic acid or GSK256073), which reduced IL-6 and CCL2 release without affecting cell viability. Our findings suggest that microglial HCAR2 activation has an antinociceptive effect, contributing to the resolution of neuropathic pain. To further explore its cell-specific role, we generated a microglia-specific *Hcar2* knockout mice (*Hcar2*^{flax/flax};Tmem119^{CreER}) for future experiments.

Keywords: Neuropathic pain;HCAR2;Microglia

2876 - Category: NEUROIMMUNOLOGY (NI)

Trained Immunity and Innate Immune Tolerance in Microglia Affect Neuroinflammation caused by Oligomeric β -Amyloid PeptideTEIXEIRA, B.T.¹; VALERIO, R.R.²; BERNARDI, A.²; BONOMO, A.C.²; FROZZA, R.L.². 1. FIOCRUZ, NITEROI - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

A doença de Alzheimer (DA) é uma doença neurodegenerativa caracterizada por perda progressiva de memória. Nas últimas décadas, evidências acumuladas revelaram que a ativação imune inata e a subsequente neuroinflamação estéril representam características essenciais dos processos neurodegenerativos que não apenas caracterizam, mas também contribuem para o desenvolvimento da DA. Consequentemente, pesquisadores têm se concentrado intensamente na compreensão dos mecanismos imunomediados e na determinação de como processos imunológicos específicos podem ser alvos de intervenção terapêutica na DA. Recentemente, demonstrou-se que as células imunes inatas podem desenvolver uma resposta de memória imunológica, aumentando ou reduzindo a resposta inflamatória após a exposição a um estímulo secundário. Isso levanta uma questão interessante sobre como o desenvolvimento da memória imune inata na microglia influencia os processos degenerativos associados à DA. Culturas organotípicas de hipocampo foram estimuladas com IL-1 β uma vez, para induzir imunidade treinada, ou repetidamente, para induzir tolerância imune inata, seguidas de exposição a oligômeros de β -amiloide (A β Os). Avaliamos os níveis de citocinas (TNF- α , IL-6), a expressão de Iba-1 e GFAP, e a ativação da via NF κ B. Nossos resultados indicaram que a estimulação repetida com IL-1 β levou a uma redução gradual de TNF- α e IL-6, uma tendência também observada com estimulação única. Curiosamente, fatias que foram primeiramente estimuladas com IL-1 β e então expostas a A β Os mostraram uma liberação reduzida dessas citocinas em comparação com fatias que foram expostas a A β Os sem estimulação prévia de IL-1 β . A expressão de Iba-1 aumentou após estimulação única de IL-1 β ou exposição a A β Os. Entretanto, a estimulação repetida com IL-1 β seguida de exposição a A β Os resultou em uma expressão diminuída de Iba-1. Não observamos nenhuma alteração na expressão de GFAP ou na ativação de NF κ B. Embora nossas descobertas sejam preliminares, elas sugerem que a memória imune inata pode ser aproveitada para reduzir a resposta inflamatória na DA.

Keywords: Alzheimer's disease;microglia;innate immune memory.

2910 - Category: NEUROIMMUNOLOGY (NI)

GENETIC CORRELATION BETWEEN ANXIETY-DEPRESSION SYMPTOMS AND ASTHMA IN CHILDHOOD: A COMMUNITY-BASED SAMPLE FROM SALVADOR

SANTOS, A.O.M.; BRITTO, G.D.S.G.; DALTRO, J.D.P.; AMARAL, E.H.B.; PEDRO, R.B.D.S.; LESSA, A.C.M.; DE JESUS, T.S.; MARINHO, A.I.; NOVAES, S.D.; DE SOUZA, A.C.L.; DE SOUSA, E.S.C.; OLIVEIRA, P.R.S.. UNIVERSIDADE FEDERAL DA BAHIA (UFBA), SALVADOR - BA - BRASIL.

Several lines of evidence indicate correlations between behavioral conditions and asthma, at phenotypic and genetic levels. However, mechanisms shared by these traits need further understanding. This study investigated the genetic correlation between Symptoms of Anxiety and Depression "(SAD)" and Asthma Symptoms "(SAM)" in admixed Latin American children from the SCAALA Project (Salvador, Brazil). After genotyping, imputation, and quality control, the dataset included 1,245 individuals for SAM, 1,190 for SAD, and ~12 million single nucleotide polymorphisms "(SNPs)". SAD was identified using the "anxiety/depression" subscale of the Child Behavior Checklist; SAM was identified via the ISAAC questionnaire. GWAS were conducted for SAD and SAM using multivariate linear and logistic regression, respectively, adjusting for seven principal components as covariates. Genetic Correlation "(rg)" was estimated by LDSC, and cross-trait meta-analysis "(CTMA)" was performed with mvGWAMA. Six SNPs were significantly associated with SAD: rs149627175 ($p = 1.3 \times 10^{-8}$; $B = 6.4$) and rs114859592 ($p = 3.8 \times 10^{-8}$; $B = 6.2$) on 18q21.2; rs111433185, rs112831292, and rs76619803 ($p \sim 2.6 \times 10^{-8}$; $B = 7.4$) on 19p13.12; and rs75710504 ($p = 2.8 \times 10^{-8}$; $B = 6.3$) on 5q33.1. Two SNPs were significantly associated with SAM: rs73014190 ($p = 1.8 \times 10^{-8}$; $OR = 2.6$; 3q25.33) and rs1999071 ($p = 2.9 \times 10^{-8}$; $OR = 1.8$; 14q11.2). Analyses suggest a moderate genetic correlation ($rg = 0.46$; $p = 0.048$). CTMA highlighted two SNPs significantly associated with both traits, rs73014190 and rs75710504 (both $Z = 5.6$; $p < 5 \times 10^{-8}$), plus 21 new index loci not identified in the individual GWAS. These variants are in genes such as *ME2*, *IQCJ-SCHIP1*, *GM2A*, *CC2D1A*, and *IL27RA*, involved in nervous system function via energy metabolism, synaptic transmission, neurotransmitters levels and neuroinflammation. These findings improve understanding of the genetic mechanisms underlying SAD and SAM in Latin American children.

Keywords: behavior problems;asthma;genetic correlation.

2931 - Category: NEUROIMMUNOLOGY (NI)

Crosstalk between microglia and T lymphocytes in a preclinical model of Alzheimer's disease

SALES, G.A.M.; DE CARVALHO, P.C.S.; SILVA, Y.P.; XAVIER, F.H.D.C.; VIANNA, P.H.O.; SANTOS, E.C.; VALERIO, R.R.; SANTOS, Á.R.C.; BONOMO, A.C.; FROZZA, R.L.. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Alzheimer's disease (AD) is the most common form of dementia, affecting millions of people worldwide. It is characterized by the accumulation of amyloid- β peptide oligomers (A β Os), synaptic dysfunction, and memory loss. Additionally, it became widely recognized that neuroinflammation is an important early event in the progression of AD. Increasing evidence points to a pivotal role of immune processes in the pathogenesis of AD, with microglia playing a central role. However, is controversial how the adaptive immune system may affect the onset and progression of AD. Therefore, investigating the interactions between microglia and T lymphocytes may open new opportunities for developing effective therapeutic alternatives for AD. To gain insights into how the immune system is involved in the pathogenesis of AD, we aimed to evaluate whether microglia activated by A β Os could interact with lymphocytes in the hippocampus and how this interaction affects microglial morphology and A β O clearance. Using the hippocampal organotypic culture model exposed to A β Os, we evaluated whether total or CD4⁺ T lymphocytes derived from deep cervical or inguinal lymph nodes of naïve mice or mice immunized with MOG or OVA would influence microglial activation, morphology and A β O clearance. Immunofluorescence and confocal microscopy were used to evaluate the colocalization of microglia and CD4⁺ T lymphocytes, microglial morphology and the A β O internalization. Although our results are preliminary, they suggest that the origin of lymphocytes and their prior exposure to CNS antigens may influence their interaction with microglia following exposure to A β Os. These findings underscore the potential of the crosstalk between the CNS and the immune system to enhance our understanding of AD's pathophysiology.

Keywords: Alzheimer's disease;CD4⁺ T lymphocytes;amyloid- β peptide oligomers.

2937 - Category: NEUROIMMUNOLOGY (NI)

IN EAE ANIMALS, MODEL FOR MULTIPLE SCLEROSIS, TREATMENT WITH POSITIVE ALLOSTERIC MODULATOR OF MGLUR5 (VU0409551) PREVENTS A INCREASE IN THE NUMBER OF MICROGLIA AND DOESN'T MODIFY MORPHOLOGICAL PATTERNS AND ACTIVATION IN THIS CELLS IN THE MODEL'S REMISSION

FANTIN, J.; GOULART, M.T.; RIBEIRO, F.. FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Multiple Sclerosis (MS) is a neurodegenerative, autoimmune, inflammatory, and demyelinating disease. In this context, microglia have a fundamental role and are the primary immunocompetent cells of the central nervous system. The mGluR5 has been identified as a potential therapeutic target for neurodegenerative and neuroinflammatory diseases. Activation of mGluR5 can lead to decreased microglial activation and neuroinflammation in contexts similar to MS. The objective was to evaluate whether treatment with a positive allosteric modulator of mGluR5 could reverse changes in morphological, activation and number of microglial parameters. The EAE model was induced by subcutaneous injection containing MOG35-55 (150 μ g/100 μ l), CFA (equal volume to MOG), M. tuberculosis (4mg/mL), and intraperitoneal injection of pertussis toxin (300ng/200 μ l) on 0 and 2 dpi in female C57BL/6 mice, with approval by the ethics committee (170/2020). VU treatment (10mg/kg) was administered daily, starting from day 10 up to 28 dpi. On 28 dpi, the animals were euthanized, and the brains were collected for number, activation and morphology analysis of the cells. In the immunohistochemistry analyses with microglial staining during the remission phase (28 dpi), it was found that EN animals had an increase in microglia, and the VU treatment reversed that. Besides, it was observed that the number of microglia is influenced by the clinical score of the animals. Also, microglial morphology, such as number and length of branches, area, number of junctions and diameter, and cells activation parameters, such as IBA and CD68 fluorescence intensity, were not different between control and EAE animals. In conclusion, our data shows that during remission the VU treatment reverts the increase of microglia in EAE animals, however, it doesn't change morphology and activation parameters in this cells.

Keywords: Microglia;Experimental autoimmune encephalomyelitis;Multiple Sclerosis.

2958 - Category: NEUROIMMUNOLOGY (NI)

Strain-specific CD4⁺ T-Cell trajectories and glutamate-driven cytotoxic modulation in experimental autoimmune encephalomyelitis

ALVES, N.M.¹; ALMEIDA, R.D.N.²; TROMBETTA, B.A.²; PERON, J.P.S.²; FARIAS, A.D.S.¹. 1. UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS - SP - BRASIL; 2. UNIVERSITY OF MASSACHUSETTS CHAN MEDICAL SCHOOL, WORCESTER - ESTADOS UNIDOS DA AMERICA.

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), driven by autoreactive T cells that cross the blood-brain barrier, leading to neuroinflammation and demyelination. Experimental autoimmune encephalomyelitis (EAE) is a widely used model for MS, with C57BL/6 mice developing monophasic disease and SJL mice presenting a relapsing-remitting course similar to human MS.

Cytotoxic CD4⁺ T cells (CD4 CTLs), marked by Runx3 expression and the production of granzyme B, IFN γ , and GM-CSF, are linked to MS severity, likely by targeting oligodendrocytes. In EAE, these cells may arise from Th17 cells that infiltrate the CNS and convert into Th1-like (ex-Th17) cells, losing IL-17 while acquiring a pro-inflammatory, cytotoxic profile. Glutamate, a key neurotransmitter in cognitive processes, signals through NMDA receptors (NMDAR) expressed on human T cells can influence cytokine production and immune cell function.

To investigate the CD4 T-cell dynamics in EAE and how NMDAR signals could shape them, we analyzed CD4 CTLs and ex-Th17 cells in both EAE models across disease stages. In C57BL/6 mice, CD4 CTLs appeared early and dominated at peak disease whereas SJL mice initially exhibited a predominance of ex-Th17 cells in the CNS, followed by a later shift toward CD4 CTL enrichment. Interestingly, ex-Th17 cells persisted in the draining lymph nodes of SJL mice, suggesting their role as a reservoir for subsequent relapses. Blocking NMDAR signaling markedly reduced CD4 CTL expansion in the CNS of C57BL/6 mice, underscoring the importance of local glutamatergic pathways in driving cytotoxic differentiation. Together, these findings reveal distinct trajectories for pathogenic CD4⁺ T-cell subsets across EAE models and suggest CNS-specific signals such as glutamate may critically shape immune responses in MS.

Keywords: Neuroinflammation; Experimental autoimmune encephalomyelitis; CD4⁺ T cell cytotoxicity.

3022 - Category: NEUROIMMUNOLOGY (NI)

ASTROCYTIC PKM2 ACTS AS A METABOLIC BREAK FOR NEUROINFLAMMATION

DE ANDRADE, L.P.¹; GUIMARÃES, R.M.¹; ADJAFRE, B.L.²; SILVA, C.E.A.¹; PAIVA, I.M.³; SILVEIRA, J.R.¹; GOMES, F.I.F.¹; GUIMARÃES, F.S.¹; CUNHA, F.Q.¹; ALVES FILHO, J.C.F.¹; CUNHA, T.M.¹. 1. FMRP/USP, RIBEIRÃO PRETO - SP - BRASIL; 2. FUNDAÇÃO HEMOCENTRO/USP, RIBEIRÃO PRETO - SP - BRASIL; 3. FIOCRUZ/RJ, RIO DE JANEIRO - RJ - BRASIL.

Astrocytes are the most present glial cells in the Central Nervous System (CNS) and play a major role in the development of neuroinflammatory diseases. Given the highly energetic profile of astrocytes, these cells have the ability to alter their own metabolism to favour CNS homeostasis. Glucose oxidation is carried out mostly by glycolysis, with pyruvate catalysis and ATP formation as final reaction through activity of the enzyme Pyruvate Kinase (PK) - with the M2 isoform (PKM2) being widely described in inflammatory conditions. In anaerobic conditions, pyruvate can be used as a substrate for lactate formation, also described as important in inflammation. Since the literature demonstrates that both immune and CNS resident cells undergo metabolic changes in neuroinflammation, the aim of this work is to evaluate the role of the axis PKM2-lactate in astrocytes neuroinflammation (CEUA 1103/2022). We observed that absence of PKM2 in astrocytes (Aldh1^{creER}/PKM2^{fl/fl}) induce a worse clinical score of Experimental Autoimmune Encephalomyelitis, corroborating with increased spinal cord demyelination and higher leukocyte infiltrate. By the end of the EAE timecourse, GFAP expression on astrocytes is reduced and there is no change in IBA-1 expression on microglia. Astrocytes of primary culture were obtained from conditional knockout mice (GFAP^{cre}/PKM2^{fl/fl}) and after inflammatory stimuli we obtained higher production of inflammatory mediators (IL-6 and CXCL1) and increase of *Gfap* and *Hif1a* expression after 6h of TNF stimuli (10 ng/mL) by the knockout cells once compared to wildtype littermates. So far, we demonstrated that astrocytic PKM2 has an antiinflammatory function. For immediate next steps, we will characterize the peripheral response on the draining lymph nodes, as well as the leukocyte infiltrate of the spinal cord during EAE. In addition, we will begin to evaluate the possible role of lactate, dependent on PKM2, using conditional knockout mice (Aldh1^{creER}/LdhA^{fl/fl}).

Keywords: Astrocytes; Neuroinflammation; Immunometabolism.

3036 - Category: NEUROIMMUNOLOGY (NI)

Development of an In Vitro Model of Intracerebral Hemorrhage to Evaluate Microglial Modulation Strategies

PEREIRA, L.C.V.¹; CORREA, A.M.C.²; BALTHAR, Y.D.C.D.S.²; RABELLO, T.²; MARON-GUTIERREZ, T.²; BOZZA, F.A.¹; DO VALLE, C.Z.¹. 1. INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS, FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO OSWALDO CRUZ, FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Intracerebral hemorrhage (ICH) is a leading cause of mortality and neurological morbidity worldwide. It is characterized by vascular rupture and blood extravasation into the brain parenchyma. Secondary brain injury after ICH is linked to neuroinflammation processes, in which microglia—the resident immune cells of the central nervous system—play a central role. After ICH, the lysis of red blood cells and the release of heme trigger microglial activation and the production of pro-inflammatory cytokines, thereby contributing to neuronal damage. In this context, molecules capable of modulating microglial activation are promising candidates to reduce ICH-associated neurological deficits. In the present work, we aimed to develop an *in vitro* ICH model using BV2 microglia stimulated with heme. With this model, we could analyse the inflammatory response under different culture conditions for future testing of modulatory molecules. For that, cells were exposed to increasing concentrations of heme (10–40 μ M) for 6 hours in media containing depleted fetal bovine serum or no serum at all. Cell viability was quantified by PrestoBlue, revealing a progressive decrease in metabolism with higher heme concentrations, regardless of the presence of serum. The production of TNF- α , IL-1 β , IL-6, IL-10, and CXCL1/KC was quantified by ELISA. Heme stimulation induced significant TNF- α (peak at 10 μ M), IL-1 β (20 μ M), and IL-6 (40 μ M) release. IL-10 increased at higher heme concentrations, with no marked difference between culture conditions, suggesting regulatory pathway activation under stronger stimuli. CXCL1/KC was undetectable under all tested conditions. These findings validate an *in vitro* neuroinflammation model using heme as stimulus. The next phase of this project will explore the ketone body β -hydroxybutyrate (BHB) as a possible microglia modulator that could reduce inflammatory damage related to ICH.

Keywords: Neuroinflammation;Microglia;Heme.

3051 - Category: NEUROIMMUNOLOGY (NI)

EVALUATION OF THE IMPACT OF CUMULATIVE HIGH-INTENSITY INTERVAL TRAINING (3HIIT) IN THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MODEL

JÚNIOR, O.C.¹; SPERANDIO, A.C.F.¹; TEIXEIRA, L.É.A.¹; CAETANO, G.P.¹; ANDRADE, M.F.¹; CORRÊA, M.E.H.¹; VIANA, J.C.S.¹; SALVADOR, M.R.¹; PEIXOTO, M.F.D.²; PINHEIRO, L.S.¹; CARLI, A.D.P.¹; ALVES, C.C.D.S.¹; DE CASTRO, S.B.R.¹. 1. UFVJM, TEOFILÓ OTONI - MG - BRASIL; 2. UFVJM, DIAMANTINA - MG - BRASIL.

Introduction: Multiple sclerosis (MS) is an inflammatory, chronic autoimmune, and demyelinating disease of the central nervous system. Some studies have shown that physical exercises improve the clinical signs of MS. Current studies aim to determine the specific types of exercise most effective for patients with MS, providing a better quality of life. High-intensity interval training (HIIT) has been shown to be effective in various disease models. **Objective:** To evaluate cumulative high-intensity interval training (3HIIT) in the EAE model. **Material and methods:** Female C57BL/6 mice were divided into four groups (n=12 animals per group). The mice in groups 1HIIT and 3HIIT trained for six weeks were induced with the EAE protocol and remained training until the onset of EAE signs. The clinical score, food consumption, and weight of the animals were assessed up to the 21st day post-induction (dpi). **Results:** The animals in the 1HIIT group were the first to show clinical signs of EAE from the 13th dpi, followed by the EAE and 3HIIT groups that started showing clinical signs from the 14th dpi and 15th dpi, respectively. The 1HIIT group showed fewer clinical signs than the EAE group on the 16th and 17th dpi. Group 3HIIT had a lower clinical score than the EAE group from the 16th to the 21st dpi. From the 19th dpi, there was a difference between the 1HIIT and 3HIIT groups, with the 3HIIT remaining with a maximum average clinical score of 1.5 ± 0.584 on the 21st dpi. Food consumption and animal weight did not differ between the groups. **Conclusion:** According to the preliminary results of this experiment, there is a promising result concerning the 3HIIT protocol, which is superior in delaying and reducing the clinical signs of EAE.

Keywords: Multiple sclerosis;EAE;HIIT.

3054 - Category: NEUROIMMUNOLOGY (NI)

The Role of C5a-Mediated Signaling in Diabetic Neuropathic Pain

MESCHICK, C.G.; GOMES, F.I.F.; PIGATTO, G.R.; CUNHA, F.Q.; ALVES-FILHO, J.C.; CUNHA, T.M.. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL.

Neuro-immune processes across the nociceptive pathway are involved in the development of inflammatory and neuropathic pain. Evidence suggests that the C5a/C5a receptor 1 (C5aR1) axis plays a crucial role in the development of neuropathic pain. Transcriptomic analyses of post-mortem human sensory neurons from patients with painful diabetic neuropathy reveal an increase in C5aR1 expression. However, the role of C5a-mediated signaling in the development of diabetic neuropathic pain remains unknown. Therefore, the objective of this project is to investigate the role of C5a/C5aR1 signaling in the onset and development of diabetic neuropathic pain. All the experiments were approved by the Animal Experimentation Ethics Committee of the Ribeirão Preto Medical School (nº 1310/2024). Our results demonstrated that the treatment of C5aR1 allosteric inhibitor (DF3966A, 30 mg/kg, twice daily, p.o.) in streptozocin (STZ)-induced neuropathic pain decreased the mechanical allodynia in the Von Frey test (paw withdrawal threshold and frequency) and cold allodynia in the acetone test when compared to mice treated with vehicle ($p < 0.05$, two-way ANOVA with Tukey's post-hoc test). Gene expression by RT-qPCR of neuroinflammatory markers (*C5ar1*, *Cx3cr1*, *Aif-1*, *TNFA*, *Il-1 β* and *Scn5a*) showed increase in *Tnfa* in spinal cord and sciatic nerve, an increase of *Scn5a* and *Aif-1* in dorsal root ganglia (DRG), an increase of *C5ar1* in sciatic nerve and a decrease of *Cx3cr1* in the spinal cord after STZ administration. Such changes will be further assessed after treatment with DF3966A. Collectively, these findings suggest that C5aR signaling plays a crucial role in the development of diabetic neuropathic pain, needing more investigation.

Keywords: Diabetic neuropathic pain; Complement system; C5aR1.

3110 - Category: NEUROIMMUNOLOGY (NI)

THERAPEUTIC EFFECTS OF PRANLUCASTE ON DEMYELINATION, NEUROINFLAMMATION, AND SYNAPTOPATHY IN AN EXPERIMENTAL MODEL OF MULTIPLE SCLEROSIS

DE SÁ, A.B.M.¹; PEREIRA, A.C.D.P.A.¹; HERINGER, L.D.S.²; MENDONÇA, H.R.²; MARTINS, R.S.²; ARAÚJO, S.E.S.¹.
 1. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE, CAMPOS DOS GOYTACAZES - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system characterized by inflammatory demyelination and synaptopathy, an imbalance between excitatory and inhibitory neurotransmission. Although current treatments target peripheral immunity, their effects on myelination and synaptopathy are poorly explored. The anti-inflammatory pranlucaste (PLK) has shown potential to promote remyelination and modulate anti-synaptogenic pathways. We evaluated its effects in the cuprizone (CPZ)-induced demyelination model. Swiss mice were fed ground chow supplemented or not with 0.2% CPZ for 5 weeks and treated with PLK (0.1 mg/kg, i.p.) or saline. They were assessed in the Visual Cliff test, and the visual cortex was processed for Luxol Fast Blue (LFB) histology, immunofluorescence, western blot, transmission electron microscopy, and [³H]-D-aspartate ([³H]-D-ASP) release assay, with ethical approval (protocol no. 506). Visual dysfunction was efficiently prevented by PLK. Demyelination, evaluated by LFB, levels of myelin basic protein and 2',3'-cyclic nucleotide 3'-phosphodiesterase, as well as the increase of oligodendrocyte precursors positive for platelet-derived growth factor receptor alpha, were equally prevented. Inflammation was modulated by a reduction in interleukin-6, without alteration in glial reactivity. Synaptopathy was evidenced by an imbalance between inhibitory synapses (increased gephyrin and structured inhibitory synapses) and excitatory synapses (increased GluN2A/B subunits of the N-methyl-D-aspartate receptor and postsynaptic density protein 95—PSD-95). PLK prevented both inhibitory (structured inhibitory synapses) and excitatory (GluN2B and PSD-95) imbalance. The reduction of activity-regulated cytoskeleton-associated protein was restored by PLK, while [³H]-D-ASP release and glutamate/aspartate transporter levels were elevated. These data suggest that PLK attenuates visual, inflammatory, myelin, and synaptic alterations in MS.

Keywords: Inflammatory Demyelination; Gliosis; Synaptic dysfunction.

3118 - Category: NEUROIMMUNOLOGY (NI)

Effects of Melatonin, Vitamin D, and Their Combinations in a Murine Model of Sleep Deprivation and β A1-42 Peptide-Induced Dementia

LIDIO, A.V.; MEDEIROS, E.B.; SILVEIRA, G.D.B.; FENILLI, G.P.; SOUZA, C.S.M.; DO CANTO, L.V.; SILVA, M.C.; BUDNI, J.. UNESC, CRICIÚMA - SC - BRASIL.

Alzheimer's disease (AD) is the most common cause of dementia, and sleep disturbances may precede its clinical diagnosis by years, although the mechanisms involved are not yet fully understood. This study aimed to evaluate the effects of combining memantine (Mem) and donepezil (Done) with melatonin (Mela) or vitamin D (VitD) in a murine model of paradoxical sleep deprivation (PSD) associated with β A₁₋₄₂-induced dementia. Male and female mice (3 months old) were subjected to PSD for 21 days (18h/day). On day 10, β A₁₋₄₂ or ACSF was administered intracerebroventricularly. From day 11, animals received by gavage the following combinations: Done+Mem, Done+Mem+Mela, Done+Mem+VitD, or water (control). Behavioral tests (Y-maze and radial maze) were conducted from days 21 to 27. On day 27, animals were euthanized, and the frontal cortex and hippocampus were collected for cytokine analysis (TNF- α , IL-1 β , IL-4, IL-10). The PSD+A β ₁₋₄₂ model caused short- and long-term learning deficits in both sexes. The Done+Mem+Mela combination improved long-term memory in females, while Done+Mem+VitD reversed both short- and long-term memory deficits in both sexes. The model also induced neuroinflammation, with increased TNF- α (female hippocampus), decreased IL-4 (male hippocampus), and reduced IL-10 (female frontal cortex and hippocampus). Done+Mem+Mela reduced neuroinflammation by decreasing TNF- α and IL-1 β (female hippocampus), increasing IL-4 (male frontal cortex and female hippocampus), and IL-10 (male hippocampus). Done+Mem+VitD also reduced neuroinflammation by decreasing TNF- α (hippocampus of both sexes), increasing IL-4 (male hippocampus), and IL-10 (male frontal cortex and female hippocampus). In conclusion, the combination of Done+Mem+VitD was the most effective in reversing memory impairments and reducing neuroinflammation.

Keywords: Sleep disorders;Memory;Neuroinflammation.

3155 - Category: NEUROIMMUNOLOGY (NI)

ANALYSIS OF SH2B3 OVEREXPRESSION ON NEURONS AND MICROGLIA

GRIGORIOU, M.; FERRARI, M.D.F.R.. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Developmental dyslexia (DD), affecting 5-17% of the population, is the most common specific learning disorder. The identification of 42 associated genes, including SH2B3 encoding LNK protein, has advanced molecular understanding. LNK inhibits immunometabolic pathways like JAK/STAT and regulates macrophages via c-Fms (controlling proliferation, differentiation, survival, and ROS) and modulates PI3K/Akt/Erk inflammatory pathways. Its role in microglia and neuroinflammation remains unknown.

In neurons, studies (using PC12 animal models) suggest LNK may influence neurite growth and differentiation suggesting SH2B3 may contribute to DD via: 1) cellular morphology changes or 2) microglia-mediated neuroinflammation. Our study examines LNK overexpression in human SH-SY5Y (neuronal) and C20 (microglial) cell lines, analyzing morphology, inflammatory profiles, and cellular interactions. Anti-LNK antibody standardization (Western blot/immunofluorescence) confirmed basal expression. Next, we'll assess LNK's effects on differentiation, cytokine production, and neuron-microglia communication to elucidate its DD role.

Preliminary results showed higher LNK expression in BDNF-treated versus untreated differentiated SH-SY5Y cells, suggesting potential regulation through this pathway. We are investigating how LNK overexpression affects neuronal differentiation, cytokine production, and neuron-microglia communication to elucidate potential DD mechanisms.

Keywords: LNK;Dyslexia;neuroinflammation.

3212 - Category: NEUROIMMUNOLOGY (NI)

Astrocyte Reactivity Shapes Inflammasome Activation: Influence of LPS and IL-4 Programming

DE LUCENA, V.W.L.; BASSO, A.S.; BORTOLUCI, K.R.. UNIFESP, SÃO PAULO - SP - BRASIL.

Inflammasomes are cytosolic multiprotein platforms that activate caspase-1, leading to IL-1 β and IL-18 release and pyroptosis. Although classically described in immune cells, inflammasomes are also expressed in astrocytes, which support neuronal function and participate in neuroimmune responses. Upon sensing damage or infection, astrocytes may acquire reactive profiles with neurotoxic or neuroprotective features. Given their role in CNS pathologies, understanding how astrocyte reactivity modulates inflammasome activation is essential. This study investigated the influence of reactive profiles induced by LPS or IL-4 on inflammasome functionality in primary murine astrocytes. Astrocyte-enriched cultures were treated with LPS (AST-LPS) or IL-4 (AST-IL-4) for 24 hours. AST-LPS exhibited high expression of C3, a marker associated with neurotoxic astrocytes, while AST-IL-4 preferentially expressed S100A10, a marker of neuroprotective reactivity. Pre-treatment with LPS increased ASC speck formation and IL-1 β release in response to NLRP3 (nigericin) and AIM2 (poly dA:dT) agonists, whereas IL-4 treatment reduced these responses. LPS promoted increased IL-6 secretion, while IL-4 treatment reduced this response only in the context of poly dA:dT. To investigate the role of microglia, astrocyte-enriched cultures containing 7–15% microglia were subjected to magnetic purification (pAST). Microglia depletion markedly reduced ASC speck formation, and IL-1 β release was observed only in pAST-LPS. Conversely, the addition of conditioned medium from LPS-activated microglia also induced a subtle IL-1 β release by purified astrocytes after nigericin stimulation, suggesting that soluble microglia-derived factors also modulate inflammasome activation in astrocytes. These results demonstrate that the inflammatory or anti-inflammatory programming of astrocytes alters their ability to activate inflammasomes in response to agonists, and that microglial signaling contributes to this process.

Keywords: Inflammasome;Astrocyte;Microglia.

3230 - Category: NEUROIMMUNOLOGY (NI)

Modulation of Purinergic Signaling Attenuates Neuroinflammation and Edema in Experimental Cerebral Malaria

FREITAS, R.¹; MANHÃES, L.D.S.B.¹; DE SOUZA, D.C.D.S.R.¹; DO NASCIMENTO, R.S.¹; CRUZ, M.C.²; 22/00291-4, S.M.M.F.¹; MATTOS, G.H.R.³; JUCÁ, P.M.⁴; COUTINHO-SILVA, R.⁵; ROBSON, S.C.⁶; MUNHOZ, C.D.⁷; LIMA, M.R.D.¹. 1. DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL, SÃO PAULO - SP - BRASIL; 2. RESEARCH SUPPORT FACILITIES CENTER, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL, SÃO PAULO - SP - BRASIL; 3. DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL, SP - SP - BRASIL; 4. PHARMACOLOGY DEPARTMENT INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL, SP - SP - BRASIL; 5. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ - RJ - BRASIL; 6. HARVARD MEDICAL SCHOOL, BOSTON - ESTADOS UNIDOS DA AMÉRICA; 7. PHARMACOLOGY DEPARTMENT INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL, SÃO PAULO - SP - BRASIL.

Extracellular ATP (eATP) is a potent danger signal released during tissue damage and inflammation, acting on purinergic receptors to trigger immune responses. We wondered whether in cerebral malaria (CM), a severe neurological complication modeled in mice infected with *Plasmodium berghei* ANKA, excessive eATP release in the central nervous system contributes to astrocyte and microglial activation, neuroinflammation, and blood-brain barrier dysfunction. Pannexin-1 (Panx1) channels, upregulated in activated or dying cells, mediate ATP release, while the ectonucleotidase CD39 counterbalances this process by hydrolyzing eATP, modulating inflammation. In our study, CD39^{-/-} mice exhibited worsened clinical outcomes and increased mortality, whereas P2X7^{-/-} mice, lacking a key eATP receptor, showed partial protection. Based on these findings, we investigated the therapeutic potential of purinergic signaling modulation. Treatment with probenecid, a Panx1 inhibitor, significantly improved clinical outcomes, reducing weight loss, clinical scores, and histopathological damage. Notably, brain weight was reduced in treated animals, suggesting diminished cerebral edema. Microglial morphology shifted from an activated amoeboid form to a homeostatic ramified state, and astrocyte reactivity was attenuated. Moreover, combined treatment with BBG, a P2X7 receptor antagonist, further enhanced the protective effects of probenecid, indicating a synergistic role in modulating neuroinflammation. These findings reinforce the contribution of dysregulated eATP–Panx1–P2X7 signaling to CM immunopathology and support Panx1 inhibition as a promising adjunct strategy to standard antimalarial therapy, potentially reducing brain inflammation and neurological sequelae. Supported by FAPESP (2023/07938-0; 2015/20432-8) and CNPq (303810/2018-1)

Keywords: Purinergic signaling;Neuroinflammation;Cerebral malaria.

3300 - Category: NEUROIMMUNOLOGY (NI)

Inflammation-driven IDO activation and zinc deficiency link obesity to neuroimmune dysregulation: evidence from murine and human Studies

MONTEIRO, F.B.F.; DO CARMO, Í.A.R.; PURNHAGEN, L.R.P.; BRATTI, L.D.O.S.; KRETZER, I.F.. UFSC, FLORIANÓPOLIS - SC - BRASIL.

Obesity is a chronic low-grade inflammatory condition characterized by elevated pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and C-reactive protein (CRP), which affect neuroimmune signaling and contribute to mood disturbances. Zinc (Zn) deficiency, common in obesity, exacerbates systemic inflammation and may dysregulate indoleamine 2,3-dioxygenase (IDO), a key enzyme in the tryptophan-kynurenine pathway involved in immunomodulation and neuroactive metabolite production. We investigated immunometabolic and behavioral effects of obesity combined with Zn deficiency experimentally and clinically. C57BL/6J mice (CEUA/UFSC PP00892) fed standard diet (SD), high-fat diet (HFD), or Zn-deficient HFD (HFD-ZD) for 8 weeks showed increased body weight (+30%), abdominal circumference (+25%), and leptin. Serum TNF- α rose in HFD-ZD (+40%, $p < 0.05$ vs. SD), indicating inflammation. IDO activity (kynurenine/tryptophan ratio) was unchanged, but tryptophan and kynurenine levels decreased (–35% and –30%), showing disrupted metabolism. Behaviorally, HFD-ZD mice had deficits in self-care and motivation in splash test (latency to groom: 215 ± 18 s vs. SD: 132 ± 12 s, $p < 0.01$), reversed by 14-day fluoxetine (20 mg/kg). Clinically (CAAE:24279013.7.0000.0121), 43 obese patients undergoing bariatric surgery were assessed pre- and 6 months post-op. Compared to 20 healthy controls, patients showed elevated serum IDO activity, TNF- α , and increased antidepressant use (53.5% vs. 10%). Post-surgery, CRP decreased ($p = 0.0288$), but IDO activity and TNF- α remained high, and tryptophan further declined (–21%, $p < 0.05$). These data show obesity and Zn deficiency induce a pro-inflammatory state that disrupts tryptophan metabolism and sustains neuroimmune dysfunction, underlying depressive symptoms. Importantly, weight loss alone does not fully reverse these changes, underscoring the need for targeted immunometabolic interventions to address mood disorders in obesity.

Keywords: Neuroinflammation; Immunometabolism; Cytokines.

3310 - Category: NEUROIMMUNOLOGY (NI)

Targeting sepsis-induced brain dysfunction and its cognitive sequelae with metformin

ALVES, R.F.; DOS SANTOS, P.D.M.A.; APRIGIO, F.; TERRA, E.; CORDEIRO, A.D.F.; REIS, P.A.; COELHO, M.G.P.. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Sepsis is a dysregulated host response to infections by different pathogens, often leading to post-sepsis syndrome with immune, metabolic, and organ dysfunctions. The central nervous system is one of the first affected sites, clinically manifesting as sepsis-associated encephalopathy. Around 40% of patients develop acute hyperglycemia during sepsis, likely due to interactions among cortisol, catecholamines, and cytokines, which contribute to metabolic disorders, insulin resistance, neuroinflammation, and cognitive decline. This study aims to understand how sepsis induces neuroinflammation and cognitive impairment and to evaluate Metformin (MET) as a therapeutic strategy. Male C57BL/6 mice (CEUA 029-2022) were subjected to cecal ligation and puncture (CLP) under anesthesia, with cecum ligated below the ileocecal valve and punctured four times using an 18G needle. Animals received antibiotics at 6, 24, and 48 h post-surgery and were scored for specific clinical signs (present = 1, absent = 0) to determine sepsis severity (score 1–12). MET (50 mg/kg) was administered 1 h before CLP and at 6, 24, and 48 h post-CLP. Cognitive performance was assessed by the Morris Water Maze (MWM): animals explored a pool for 60 s over 4 days to locate a submerged platform; latency was recorded daily. On day 5, the platform was removed, and time spent in the target quadrant (60 s) was measured. MET-treated mice showed 45% lower glycemia 6 h after CLP and 44% lower clinical scores at 24 h, indicating milder sepsis and better prognosis, reflected in higher survival. In the MWM, MET-treated animals demonstrated consistent learning, reduced latency to find the platform, and increased time in the target quadrant compared to untreated CLP controls. These findings suggest Metformin may attenuate sepsis severity and its cognitive consequences by modulating metabolic and inflammatory pathways.

Keywords: Metformin; Insulin resistance; Sepsis.

3323 - Category: NEUROIMMUNOLOGY (NI)

IMMUNE REGULATION IN PARKINSON'S DISEASE: ANALYSIS OF PLASMA CYTOKINES AND CORRELATION WITH DISEASE PROGRESSION

VIANA, L.D.S.¹; BRITO, D.V.¹; CATÃO, C.L.D.S.²; FERREIRA, G.B.M.³; DE OLIVEIRA, E.Y.C.⁴; DE ANDRADE, B.W.¹; DOS SANTOS, F.A.²; DE LIMA, A.B.⁴; PEREIRA, D.D.S.²; COLETA, M.V.D.³; FANTIN, C.¹; DA COSTA, A.G.². 1. UNIVERSIDADE DO ESTADO DO AMAPÁ (UEA), MANAUS - AM - BRASIL; 2. UNIVERSIDADE FEDERAL DO AMAPÁ (UFAM), MANAUS - AM - BRASIL; 3. HOSPITAL UNIVERSITÁRIO GETÚLIO VARGAS (HUGV), MANAUS - AM - BRASIL; 4. FUNDAÇÃO HOSPITALAR DE HEMATOLOGIA E HEMOTERAPIA DO AMAPÁ (HEMOAM), MANAUS - AM - BRASIL.

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss and α -synuclein protein accumulation. Aggregates of α -synuclein can activate microglia, triggering neuroinflammation and the release of pro-inflammatory cytokines and toxic mediators. In addition, immune response may precede motor symptoms by several years, associated with disease progression. In this context, identifying peripheral inflammatory markers may help clarify PD pathophysiology and reveal prognostic biomarkers.

Objectives: This study evaluated plasma levels of IL-1 β , IL-6, IL-10, IL-12p70, and TNF in PD patients, correlating them with clinical severity. **Methods:** 36 patients were stratified by Hoehn & Yahr stages into mild (stages 1–2) and severe (stages 3–5) groups. Cytokine concentrations were measured by cytometric bead array (CBA) using flow cytometry (FACS Canto II) and analyzed with FCAP-Array™ software. Statistical analyses were performed using GraphPad Prism (v10.2.3). **Results:** TNF and IL-10 levels were significantly reduced in both mild ($p < 0.05$; $p < 0.01$) and severe ($p < 0.01$; $p < 0.05$) PD groups compared to controls. IL-6 was significantly reduced only in the severe group ($p < 0.05$). IL-1 β showed a decreasing trend without statistical significance. In contrast, IL-12p70 levels were significantly increased in both mild ($p < 0.01$) and severe ($p < 0.05$) PD groups. **Conclusion:** The reduction of TNF, IL-6, and IL-10, especially in advanced stages, suggests peripheral immune dysregulation, possibly related to immune exhaustion or impaired mechanisms. Nonetheless, the increase in IL-12p70 indicates sustained innate immune activation and possible Th1 polarization, suggesting immune reprogramming potentially triggered by neuronal antigens such as α -synuclein.

Keywords: Parkinson's disease; Neuroinflammation; Peripheral immune response.

3346 - Category: NEUROIMMUNOLOGY (NI)

Mechanisms associated with nociceptive alterations caused by *Leishmania amazonensis* infection in C57BL/6 mice.

OLIVEIRA, G.D.S.M.D.¹; DOS SANTOS, H.P.²; MANHÃES, N.C.D.S.²; PEREZ, G.G.³; CLARKE, J.H.R.⁴; GUEDES, H.L.D.M.¹. 1. IOC - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. ICBCCF - UFRJ, RIO DE JANEIRO - RJ - BRASIL; 4. ICB - UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis is a group of non-contagious infectious diseases caused by the *Leishmania* parasite, which is predominant in tropical and subtropical regions. These diseases can manifest in various clinical forms, as the cutaneous one is the most prevalent in Brazil, mainly caused by *Leishmania braziliensis*, *L. amazonensis* and *L. guyanensis*, is characterized by inflamed and ulcerated lesions, usually painless. Based on our group's previous findings of IL-1 β -dependent hyperalgesia in C57BL/6 (B6) Wild Type (WT) mice. The present study aims to evaluate the role of both T and B cells in the hypersensitization of B6 mice infected with *L. amazonensis*. To this, groups of transgenic mice lacking T CD4⁺(CD4^{-/-}), T CD8⁺(CD8^{-/-}), or mature B cells (μ MT) were infected in the right hind paw pad. Lesion sizes were monitored, and their thermal sensitivity was evaluated by the hot plate test. In the μ MT mice, there was no difference in lesion development compared to WT mice. However, in the chronic phase, these mice showed a longer reaction time to thermal stimuli, which indicates reduced sensitization. The CD4^{-/-} group exhibited greater lesion progression than WT mice in the chronic phase but showed no difference in the hot plate test, indicating similar sensitization to WT mice. Finally, the late chronic phase of CD4^{-/-} and CD8^{-/-} mice were compared, revealing an opposite lesion profile and a partial reduction in thermal hyperalgesia in CD8^{-/-} mice. Von Frey's test was performed to assess mechanical sensitization. There was a significant difference in sensitization between groups, as the CD8^{-/-} mice challenged paws were slightly more sensitive to touch than the control paw and the CD4^{-/-} mice paws. In summary, data show that mature B cells and CD8⁺ lymphocytes can play a role in hypersensitization associated to *L. amazonensis* infection. Overall, further experiments are needed to fully determine the dynamics between lymphocytes and antibodies – mainly IgM – in nociceptive alterations.

Keywords: Hyperalgesia; Leishmaniasis; Lymphocytes.

3361 - Category: NEUROIMMUNOLOGY (NI)

Changes in the central nervous system in sepsis-associated encephalopathy and its cognitive sequelae: a proteomic study

TERRA, E.; CORDEIRO, A.D.F.; DA LUZ, C.S.; PEREIRA, L.M.M.; DOS SANTOS, P.D.M.A.; ALVES, R.F.; TAKEUTI, V.Y.; APRIGIO, F.; REIS, P.A.. STATE UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Sepsis is a critical global public health issue, characterized by a dysregulated host immune response to life-threatening infection, leading to organ dysfunction and high mortality rates. Patients commonly suffer from Sepsis-Associated Encephalopathy (SAE), an acute neurological dysfunction often progressing to long-term cognitive deficits like memory loss and learning difficulties. Its molecular mechanisms in the central nervous system (CNS) are poorly understood. This study aimed to investigate and identify, via high-resolution proteomics, alterations in CNS protein profiles and biological pathways related to cognitive dysfunction in experimental sepsis models. We used a polymicrobial sepsis model by cecal ligation and puncture (CLP) in C57BL/6 mice, mimicking moderate-to-severe human sepsis (50% mortality at 24h). Biochemical analyses revealed metabolic dysregulation, including dyslipidemia with increased LDL (~550 mg/dL; $p < 0.05$) and triglycerides (~120 mg/dL; $p < 0.01$), reduced HDL (~130 mg/dL; $p < 0.05$), and liver injury (increased ALT ~1350 U/L; $p < 0.05$, and AST ~1250 U/L; $p < 0.05$). Systemic oxidative stress was exacerbated, with increased TBARS and total thiol depletion within the first 24h. Glycemic dynamics showed initial hyperglycemia followed by severe persistent hypoglycemia up to 72h. Neurocognitive deficits were significant: at 7 and 15 days, the freezing test revealed substantial impairments in short and long-term memory ($p < 0.05$). At 15 days, the Morris Water Maze (MWM) confirmed clear spatial memory impairment ($p < 0.01$). Preliminary ELISA analyses indicated serum cytokine alterations (IL-6, IL-1, TNF- α , MCP-1, CXCL-1) at 6 and 24h post-CLP. The CLP sepsis model reproduced systemic aspects (metabolic dysregulation, organ damage, oxidative stress) and induced pronounced neurocognitive deficits. These results, similar to human pathophysiology, highlight SAE's complexity and validate the model for future proteomic investigations.

Keywords: Sepsis-Associated Encephalopathy; Proteomics; Sepsis.

3387 - Category: NEUROIMMUNOLOGY (NI)

SARS-CoV-2 Spike Protein Disrupts Carnitine-Dependent Fatty Acid Oxidation and Promotes Microglial Inflammatory Activation

PEREIRA, H.G.; DE OLIVEIRA, M.D.A.; BELTRÃO, G.S.; BLANK, M.; RODRIGUES, T.S.; PINTO, A.R.. UFSC, FLORIANÓPOLIS - SC - BRASIL.

Introduction: The severe acute respiratory syndrome virus 2 (SARS-CoV-2), the causative agent of the largest public health crisis of the 21st century, COVID-19, has been widely described as causing systemic impacts. In addition, consequences on the central nervous system (CNS), such as neuroinflammation and neurodegeneration, have been highlighted. One of the main mechanisms proposed to explain the neuropathogenesis of COVID-19 is the exacerbated activation of microglial cells due to their interaction with the Spike (S) glycoprotein of SARS-CoV-2, which results in a chronic pro-inflammatory environment in the CNS. **Aim:** To identify metabolic alterations associated with neuroinflammation induced by the SARS-CoV-2 S glycoprotein in vitro. **Methods and results:** Mouse immortalized microglial cells (BV-2) were stimulated with S protein (3,0 $\mu\text{g/mL}$) for 60 hours to evaluate cell activation and a pro-inflammatory phenotype by Flow cytometry. In order to understand the immunometabolic aspects associated with the activation of microglial cells by the S protein, this study conducted a metabolomic analysis using a liquid chromatography system coupled to a high-resolution mass spectrometer. The results show that the activation of microglial cells with S glycoprotein induces a negative modulation in carnitine metabolism, resulting in the reduction of beta-oxidation of fatty acids, increased oxidative stress and the polarization of microglial cells towards a pro-inflammatory phenotype as observed by an increase in CD40, CD86, CD206 and CD163 markers. **Conclusion:** In this manner, this study points to a possible involvement of this metabolic pathway in neuropathogenesis related to SARS-CoV-2 infection.

Financial support: CAPES; CNPq; FAPESC

Keywords: SARS-COV-2; Microglia; Immunometabolism.

3391 - Category: NEUROIMMUNOLOGY (NI)

Effects of the SARS-CoV-2 Spike Protein on the Lipid Metabolism of Microglial Cells

BELTRÃO, G.S.; DE OLIVEIRA, M.D.A.; PEREIRA, H.G.; BLANK, M.; RODRIGUES, T.S.; PINTO, A.R..
UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRASIL.

INTRODUCTION: SARS-CoV-2, the agent of COVID-19, represents the most significant public health crisis of the 21st century. Beyond its systemic manifestations, growing evidence points to relevant neurological effects, including processes of neuroinflammation and neurodegeneration. One proposed hypothesis for the neuropathogenesis of the infection involves the exacerbated activation of microglia, triggered by interaction with the viral Spike (S) glycoprotein, leading to a persistent pro-inflammatory state in the central nervous system. **AIM:** To identify lipid metabolic alterations associated with neuroinflammation induced by the SARS-CoV-2 S glycoprotein in vitro. **METHODOLOGY:** This study explored microglial activation by analyzing cytokine levels and lipidomic profiles in BV-2 cells stimulated with the SARS-CoV-2 S glycoprotein (3,0 µg/mL) for 60 hours. **RESULTS:** Flow cytometry revealed increased production of TNF-α, while mass spectrometry identified eight modulated metabolites. MS/MS fragmentation analyses detailed changes in lipids from the sphingolipid, glycerophospholipid, and glycerolipid classes. **CONCLUSION:** The data indicate that microglial stimulation by the SARS-CoV-2 S glycoprotein leads to specific alterations in the lipid profile. These metabolites may be functionally involved in mediating the inflammatory response, suggesting a potential role in the induction and maintenance of COVID-19-associated neuroinflammation.

Financial support: CAPES; CNPq; FAPESC

Keywords: SARS-COV-2;Microglia;Immunometabolism.

3437 - Category: NEUROIMMUNOLOGY (NI)

Adrenergic signaling via β2AR modulates astrocyte responses during inflammatory conditions

SILVA, E.D.S.¹; SANTOS, W.G.S.²; VIEIRA, P.M.M.D.M.²; BASSO, A.S.¹. 1. FEDERAL UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. STATE UNIVERSITY OF CAMPINAS, SÃO PAULO - SP - BRASIL.

Central nervous system (CNS) diseases such as Multiple Sclerosis and Alzheimer's disease are associated with high morbidity. In several cases of neurodegenerative disease, patients often experience rapid neurological decline and limited therapeutic options. Resident CNS cells undergo phenotypic and functional changes in response to inflammatory and degenerative stimuli, playing a pivotal role in disease pathogenesis. Although the CNS has mechanisms to limit inflammation, these can be impaired during disease progression. In this context, we investigated how adrenergic signaling via B2-adrenergic receptors (B2AR) in astrocytes modulates inflammatory responses and glutamate metabolism. For this, primary culture of astrocytes and microglia were prepared, and last, microglia were activated to obtain microglia-conditioned medium. We showed B2ARs are functionally expressed in astrocytes, as evidenced by increased CREB phosphorylation after receptor activation. Using a microglial inflammatory model (conditioned medium from LPS-activated microglia (MCM)), we observed changes in EAAT1 expression at 6, 12, and 24h. MCM contained high levels of TNF-α, and direct TNF-α stimulation for 6h indeed reduced EAAT1 protein expression, which was reversed to basal levels by B2AR agonist pre-treatment in a canonical signalling pathway-dependent manner. In the same context, TNF-α increased CCL2 production at 6 and 24h, which was attenuated by B2AR activation. Since glutamate metabolism is linked to energy metabolism, we found that the microglia-inflammatory model hampered glycolytic capacity, glycolysis-derived ATP production, and lactate release. Additionally, mitochondrial dynamics were also disrupted. Notably, β2AR signalling restored glycolytic function, ATP and lactate levels, and mitochondrial potential. Collectively, our data indicate that adrenergic signaling via B2AR enables astrocytes to sustain their physiological roles despite exposure to damaging inflammatory environments.

Keywords: Sympathetic nervous system;glutamate metabolism;glycolytic flux.

3467 - Category: NEUROIMMUNOLOGY (NI)

The role of CD39 enzyme in cerebrovascular alterations induced by sepsis.

CARVALHO, L.D.A.¹; DA SILVA, M.S.P.¹; DE MORAES, B.P.T.²; DE ALBUQUERQUE, C.F.G.²; FONSECA, R.J.C.¹; COUTINHO-SILVA, R.¹; SAVIO, L.E.B.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Sepsis is characterized by an exacerbated immune response of the host to an infection, resulting in multiple organ dysfunction. Vascular alterations and endothelial activation are key features, causing disruption of the endothelial barrier and tissue inflammation with infiltration of immune cells. In the central nervous system (CNS), the blood-brain barrier (BBB) dysfunction contributes to sepsis-associated encephalopathy. Activated endothelial cells can release damage-associated molecular patterns (DAMPs), such as ATP, which activates purinergic receptors or is hydrolyzed by ectonucleotidases such as CD39, which regulate ATP- induced inflammatory responses. The aim of this study was to investigate the role of purinergic signaling, particularly, NTPDase1/CD39, in cerebrovascular alterations associated with sepsis. In vitro, bEnd.3 cells were stimulated with LPS (100 or 1000 ng/mL). In vivo, sepsis was induced by cecal ligation and puncture (CLP) in wild-type (WT) and CD39-deficient (CD39^{-/-}) mice. After 24 hours, cortex, hippocampus, and serum were collected. LPS stimulation reduced CD39 protein expression and enzymatic activity in vitro, without affecting purinergic receptor levels. CD39 inhibition (with POM-1, 10 µM and AR67156, 100 µM) enhanced LPS-induced mRNA expression of endothelial markers (ICAM-1, VCAM-1, CXCL-1), especially VCAM-1 and CXCL-1. CXCL-1 quantification by ELISA also indicated an increase with LPS, which was further enhanced by CD39 inhibition. In vivo, septic CD39^{-/-} mice showed higher ICAM-1 and CXCL-1 mRNA expression in the brain, as well as elevated CXCL-1 protein levels in brain and serum compared to septic WT mice. In addition, septic CD39^{-/-} mice revealed increased leukocyte adhesion, assessed by intravital microscopy analysis, and a more pro-coagulant profile. These findings indicate that CD39 plays a protective role in modulating endothelial activation, leukocyte adhesion, and cerebral microvascular inflammation during sepsis.

Keywords: Sepsis;CD39;Inflammation.

3474 - Category: NEUROIMMUNOLOGY (NI)

HIGH-FIBER DIET MODULATES MICROBIOTA, METABOLISM, MOTOR PERFORMANCE, AND NEUROINFLAMMATION IN KNOCKIN ZQ175 MICE OF HUNTINGTON'S DISEASE

OLIVEIRA, J.D.S.B.¹; SILVA, N.C.²; FANTIN, J.²; MACIEL, E.M.A.²; BARBOSA, C.E.A.²; SANTOS, L.G.P.²; GOULART, M.T.²; BARAHUNA FILHO, P.F.R.²; RIBEIRO, R.D.S.²; RIBEIRO, F.². 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. UFMG, BELO HORIZONTE - MG - BRASIL.

Introduction: Huntington's disease (HD) is a severe, autosomal dominant neurodegenerative disorder caused by expansion of CAG repeats in the *HTT* gene, producing a toxic gain-of-function protein. There is no disease-modifying treatment, and functional limitations progress to death about 20 years after motor diagnosis. In addition to neurological symptoms, there is evidence of metabolic, inflammatory, and gut dysbiosis alterations in HD. Considering the potential of dietary fiber to modulate the gut-brain axis, this study evaluated the impact of diets with different fiber contents on microbiota, metabolic, motor, and neuroinflammatory parameters in *knockin* zQ175 HD mice. **Methods:** WT and zQ175 *knockin* mice were fed control, high-fiber, or fiber-free diets from weaning to 15 months. Body weight, adiposity index, fasting blood glucose (~6 h), behavior (open field, rotarod), and immunofluorescence for microglia (Iba1/CD68) and astrocytes (S100β) in the striatum were assessed. **Results:** The high-fiber diet better preserved lean mass in HD mice, while the fiber-free diet suggested greater loss. At 15 months, only high-fiber groups showed fasting glucose significantly below 200 mg/dL. In the open field, no relevant differences were observed in spontaneous locomotor/exploratory behavior. At 10 months, zQ175 mice had motor deficits in the rotarod, prevented by the high-fiber diet. Total microglial numbers were similar, but CD68 intensity was lower in HD-control vs. WT-control and restored by high fiber, suggesting possible improvement in microglial phagocytic capacity, to be confirmed in further analyses. Astrocytosis was more pronounced in HD, especially without fiber. Over time, the high-fiber diet was associated with increased cultivable *Enterobacteriaceae* in feces. **Conclusion:** The high-fiber diet mitigated motor deficits, preserved metabolic parameters, and modulated neuroinflammation in *knockin* HD mice, suggesting potential as a non-pharmacological intervention.

Keywords: Huntington's disease;Microbiota-gut-brain axis;Dietary fiber.

3481 - Category: NEUROIMMUNOLOGY (NI)

The kynurenines metabolites as a promise Biomarks of Hansen's Neurophaty.SANTANA, R.C.; RAHMAN, A.U.; PEREIRA, M.M.D.P.; SARNO, E.N.; RODRIGUES, M.M.J.; PINHEIRO, R.O..
INSTITUTO OSWALDO CRUZ IOC/ FIOCRUZ MANGUINHOS, RIO DE JANEIRO - RJ - BRASIL.

Leprosy is an infectious disease that affects the dermal tissue and the peripheral nervous system (PNS). In nerve fibers, the infectious agent *Mycobacterium leprae* colonizes Schwann cells and the inflammatory environment, mostly associated with reactive episodes, resulting in neural damage. The resulting disabilities have a tremendous impact on patient's life. The treatment is effective in preventing physical disabilities, but the use depends on an accurate and immediate diagnosis, which is difficult for being clinical and requires additional support tests. The metabolization of tryptophan by the enzyme indoleamine 2,3-dioxygenase (IDO-1) leads to the production of neuromodulators also known as kynurenines which act in the central nervous system, where their imbalance leads to neurodegenerative diseases. However, there is limited research related to the PNS. Previous work by our group demonstrates that multibacillary patients have high IDO-1 expression in serum and lesions and that mycobacteria and their cellular fractions lead to enzyme activation. In this study, we evaluated the production of kynurenine pathway metabolites in leprosy patients and their relationship with the development of Leprosy nerve damage. Patients with dermatological leprosy (BT, BL, and LL) and reactive (RR and ENH) showed high expression of Quinolinic Acid (QUIN), Tryptophan, and Xanthurenic Acid (XA) while they had lower expression of 2-Oxadipic acid compared to patients with neurological alterations (Pure Neural and Other Neuropathies). Reactive leprosy patients also had lower expression of Anthranilic Acid. Logistic regression analyses indicated that QUIN, XA and NAD⁺ are related to the development of neural damage, while the ROC curve showed that QUIN had a sensitivity, specificity, and accuracy of 74.8%, 78.5%, and 95.5%, respectively, indicating that this metabolite could be used as a possible biomarker for the Leprosy neural damage.

Keywords: Leprosy neuropathy; Indoleamine 2,3-dioxygenase; kynurenines.

3491 - Category: NEUROIMMUNOLOGY (NI)

Involvement of CD39 in Long-Term Brain Dysfunction in sepsis-surviving miceDA SILVA, M.S.P.; ALVES, V.S.; RODRIGUES, F.C.; DE FIGUEIREDO, C.P.; COUTINHO-SILVA, R.; SAVIO, L.E.B..
UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Sepsis is a severe organ dysfunction caused by an unregulated inflammatory response to infection, with high incidence and mortality rates, and is one of the leading causes of death in intensive care units. Sepsis-associated encephalopathy (SAE), present in up to 70% of cases, manifests as delirium and can progress to coma. The resulting inflammation can activate glial cells, which release cytokines and reactive oxygen species, causing neuronal damage. ATP (adenosine triphosphate) acts as a danger signal when released into the extracellular environment. The enzyme CD39 hydrolyses ATP into ADP and then into AMP, and in the context of sepsis, CD39 has been shown to play a protective role by attenuating systemic and neuroinflammatory inflammation in sepsis acute models. This project investigates the role of CD39 in long-term cognitive and brain alterations in sepsis-surviving mice. Wild-type (WT) and CD39-deficient (CD39^{-/-}) male mice were used, in which sepsis was induced by the cecal ligation and perforation (CLP) method (CEUA Approval: 056/24). After 13 days, long-term memory was assessed by the novel object recognition test (NORT), and IL-1 β , IL-6, and BDNF production in the cortex and hippocampus was analysed by ELISA. The septic, WT and CD39^{-/-} animals, showed cognitive impairment 13 days after sepsis induction. Both septic animals showed increased IL-1 β and IL-6 production in the cortex and hippocampus, with a significant increase in these cytokines observed in the cerebral cortex of CD39^{-/-} mice compared to WT mice. Regarding BDNF, we observed a significant decrease in the cortex and hippocampus of septic CD39^{-/-} mice when compared to septic WT mice. The survival curve showed a decrease in the survival rate of septic CD39^{-/-} mice compared to septic WT mice. Together, these initial data suggest a role for the CD39 enzyme in long-term brain alterations induced by sepsis.

Keywords: Sepsis ; Sepsis-associated encephalopathy; CD39 enzyme.

3494 - Category: NEUROIMMUNOLOGY (NI)

Impact of SARS-CoV-2 Infection on Purinergic Receptor Expression and Neuroinflammatory Responses in K18-hACE2 Mouse Brain

DOS SANTOS, E.D.P.P.¹; CREPALDI, L.D.¹; DA SILVA, M.S.P.¹; BARBOSA, N.C.¹; RODRIGUES, F.C.¹; LIMA, L.R.¹; ARAUJO, H.P.M.¹; COLODET, L.C.¹; TEMEROZO, J.²; BOU-HABIB, D.C.²; DE ARRUDA, L.B.¹; DE FIGUEIREDO, C.P.¹; COUTINHO-SILVA, R.¹; SAVIO, L.E.B.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a significant global impact, with millions of reported deaths. Although the respiratory system is the primary target, evidence indicates that infection can also affect the central nervous system, leading to neurological disorders, neuropsychiatric manifestations, and cognitive impairments. The underlying mechanisms of these alterations are not yet fully understood, and elucidating them is essential for the development of therapeutic strategies that minimize the neurological impact of the disease. In this study, we used K18-hACE2 transgenic mice, which express the human ACE2 receptor under the cytokeratin-18 promoter, as an experimental model to evaluate the role of purinergic signaling in brain alterations induced by infection. Animals were divided into four groups: mock control, control treated with Brilliant Blue G (BBG, a P2X7 receptor antagonist), SARS-CoV-2-infected, and infected treated with BBG. Infection was performed intratracheally with 30 µL containing 2×10^4 PFU of SARS-CoV-2, while BBG was administered intraperitoneally 1 day before and 3 days after infection. The infection model was conducted for 7 days, and only after this period were brain tissues collected for analysis. Increased expression of P2Y2 and P2Y6 receptors, decreased expression of P2Y12, and upregulation of pro-inflammatory genes (IL-1 β , IL-6, TNF- α , IFN- γ , GFAP, and interferon-stimulated genes) were observed in the hippocampus and cerebral cortex, measured by real-time PCR. In addition, activation of caspase-1, caspase-3, gasdermin D, and ZBP-1 was detected, indicating the triggering of cell death pathways. These findings suggest that purinergic signaling plays a relevant role in the pathophysiology of SARS-CoV-2-induced brain alterations.

Keywords: Neuroinflammation;Purinergic signaling;SARS-CoV-2.

3523 - Category: NEUROIMMUNOLOGY (NI)

THE USE OF MOLECULAR MODELING FOR DEVELOPMENT OF NEURAL BIOMODELS TO STUDY OF AUTISM

LIONE, V.D.O.F.¹; RIBEIRO, B.S.A.¹; ALBUQUERQUE, L.H.P.¹; TRINDADE, P.²; SODERO, A.C.R.². 1. FF-UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. FF, RIO DE JANEIRO - RJ - BRASIL.

Autism Spectrum Disorder (ASD) is a multifactorial neurodevelopmental condition characterized by deficits in social communication, interaction, and repetitive behavioral patterns. Recent studies have highlighted not only an increased microglia population in various brain regions but also the central role of inflammatory markers in its pathophysiology. This neuroinflammation is mediated by an exacerbated activation of microglia and astrocytes, as well as alterations in cytokines. Aim is to understand the interactions between extracellular matrix (ECM) proteins and hydrogel (PVA and NaCMC), assisting in the development of new biomodels for autism research. **Methods and Results:** The three-dimensional *in vitro* biomodels were developed using monocyte-derived microglia (MiDMs) cultured in biomimetic hydrogels functionalized with ECM components to simulate the altered neuronal microenvironment. A blind molecular **docking** study was performed using the Gold program to analyze PVA 2 monomers with tenascin C. The analysis revealed a notable superiority of the PVA 2 ligand over PVA 3. PVA 2 demonstrated higher **docking** scores, with an average of 50.15 and a maximum score of 61.98, suggesting a more energetically stable interaction. The PVA 3 achieved an average of only 45.45 and a maximum of 50.82. The main advantage of PVA 2 lies in its ability to form hydrogen bonds with amino acid residues at the tenascin C binding site, a contribution completely absent in PVA 3's interactions. **Conclusion:** Based on the molecular **docking** results, the PVA 2 compound is a considerably more potent ligand for tenascin C than PVA 3. Its higher binding affinity, mainly attributed to its ability to establish hydrogen bonds, qualifies it as a more promising conformation for the hydrogel composition. Although hydrogen bond stability still represents a challenge to be overcome, it is expected that this model ECM proteins, will provide a relevant platform to study neuroinflammation in the context of ASD.

Keywords: autism;Molecular Modeling;extracellular matrix.

2871 - Category: TRANSPLANTATION AND IMMUNOGENETICS (TR)

UNCOVERING GENETIC AND IMMUNOLOGIC MECHANISMS ASSOCIATED WITH HARMFUL USE OF ALCOHOL IN ADMIXED LATIN AMERICANS

AMARAL, E.H.B.¹; SANTOS, A.O.M.¹; BRITTO, G.D.S.G.¹; DE SOUZA, A.C.L.²; NOVAES, S.D.²; PEDRO, R.B.D.S.¹; LESSA, A.C.M.¹; DE JESUS, T.S.¹; DE SOUSA, E.S.C.²; MARINHO, A.I.¹; DALTRO, J.D.P.³; OLIVEIRA, P.R.S.¹. 1. UNIVERSIDADE FEDERAL DA BAHIA (PPGIM), SALVADOR - BA - BRASIL; 2. UNIVERSIDADE FEDERAL DA BAHIA (IBIO), SALVADOR - BA - BRASIL; 3. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL.

Harmful use of alcohol (HUA) is a major public health concern, linked to physical and psychosocial harm and straining healthcare systems. Alcohol use disorders are complex traits influenced by genetic, immune, and environmental factors, with heritability estimated at 50–60%. Although genomic studies have identified variants associated with HUA, most have focused on populations of European or Asian ancestry, limiting broader applicability. This study investigated genetic and immunogenetic mechanisms related to HUA in admixed Latin American individuals. A total of 2,840 participants from Pelotas, Brazil, were assessed using the AUDIT questionnaire to classify HUA risk. Genotyping was conducted with the Illumina HumanOmni 2.5-8v1 BeadChip, covering 2.3 million variants. Genetic ancestry was estimated using Principal Component Analysis (PCA) and ADMIXTURE. Genetic associations were tested using multivariate logistic regression. Associated variants were explored via in silico functional annotation, pathway enrichment, and gene interaction analyses. Individuals at higher risk of HUA showed greater European ancestry ($p = 0.021$). A significant association with HUA was found for an intronic variant in the CYP4B1 gene (rs1097611; $p = 4.88 \times 10^{-6}$; OR = 1.8). This and nearby variants were linked to differential CYP4B1 expression in various tissues. A suggestive association was also observed with the VAV1 gene, involved in immune responses ($p = 6.33 \times 10^{-6}$; OR = 3.16). Key biological pathways implicated include TRP channels, regulation of inflammatory mediators, and Fc-gamma receptor-mediated phagocytosis. These findings reinforce the multifactorial nature of HUA and the involvement of immune and neurotransmission pathways in substance use. Including diverse populations in genetic studies is crucial to expand understanding of the genetic basis of alcohol use disorders.

Keywords: immunogenetics; Harmful use of alcohol; Latin american.

3093 - Category: TRANSPLANTATION AND IMMUNOGENETICS (TR)

IMMUNE COMPATIBILITY ASSESSMENT VIA FLOW CYTOMETRY CROSSMATCH FOR SAFER XENOTRANSPLANTATION

BENTO, N.P.C.¹; IKEGAMI, A.²; FLATOW, E.A.²; BARBOSA, D.³; BRITO, L.A.¹; DE OLIVEIRA, V.L.²; RAIA, S.M.¹; KALIL, J.². 1. USP, SÃO PAULO - SP - BRASIL; 2. HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. A. HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Brazil has one of the largest public organ transplant systems in the world, but a shortage of compatible donors leaves over 41,000 people on waiting lists. Xenotransplantation using pig organs is a promising alternative; however, it faces significant immune barriers, including hyperacute rejection mediated by human antibodies targeting the xenoantigens DBA, Neu5Gc, and IB4, encoded by the GGTA1, CMAH, and B4GALNT2 genes, respectively. Gene editing can eliminate these antigenic targets. Triple-knockout (TKO) porcine aortic endothelial cells (pAECs) were successfully generated and provided by our collaborators at Genoma/USP. **This study investigates how the deletion of specific xenoantigens in genetically modified porcine cells modulates the human immune response, focusing on antibody recognition and immunological compatibility.**

Blood samples from wild-type (WT) pigs were collected and porcine red blood cells (pRBCs) were isolated. An adsorption protocol with human sera from sensitized patients was used to deplete anti-pig antibodies. Porcine Peripheral Blood Mononuclear Cells (PBMCs) were isolated using Ficoll density gradients or genetically modified pAECs (WT and TKO) were incubated with human sera for xeno crossmatching studies. Cells were stained for viability and surface markers.

(1) Flow cytometry confirmed successful xenoantigen knockout of relevant xenoantigens. (2) Antibodies present in human sera recognized swine antigens on PBMCs. (3) The adsorption technique effectively removed xenoreactive antibodies from human sera. (4) Deletion of xenoantigens significantly reduced IgM/IgG binding, as demonstrated by FCM-based crossmatching assays.

This experimental platform enables the functional evaluation of genetically modified porcine cells and supports the development of novel strategies to reduce xenogeneic immune responses. It holds promise for improving the compatibility and safety of xenotransplantation.

DECIT-CNPq #444097/2023-6, INCT-CNPQ-iii #408685/2024-7

Keywords: xenotransplantation; crossmatch assay; gene editing.

3101 - Category: TRANSPLANTATION AND IMMUNOGENETICS (TR)

ASSESSING XENOTRANSPLANTATION IMMUNOREACTIVITY VIA MIXED LYMPHOCYTE REACTION

FLATOW, E.A.; DE OLIVEIRA, V.L.; BENTO, N.P.C.; RAI, S.M.; KALIL, J.. HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Xenotransplantation using genetically modified pig organs represents a promising strategy to address the critical shortage of compatible human organ donors. However, despite advances in gene editing, significant immunological barriers remain. In this study, we employed Mixed Lymphocyte Reaction (MLR) assays to evaluate immune compatibility in potential xenotransplantation models. In this work, we developed flow cytometry-based analyses to compare activation, proliferation, and memory phenotypes across allogeneic and xenogeneic contexts using MLR assays.

Peripheral blood mononuclear cells (PBMCs) from human research participants and wild-type (WT) pigs were isolated by Ficoll density gradient centrifugation. Then, a one-way MLR was performed using human PBMCs (hPBMCs) in allo or porcine PBMCs (pPBMCs) in xeno settings. Stimulator cells received 25 Gy irradiation and were co-cultured with responder cells at a 1:1 ratio. hPBMCs were stained to assess T cell proliferation with CFSE. The viability and activation markers (HLA-DR, CD25, CD69), and memory/differentiation (CD45RA, CCR7, CD62L) in CD4⁺ and CD8⁺ T cell subsets were evaluated. Activation control was performed using Dynabeads CD3/CD28. Data acquisition was conducted on a BD LSRFortessa flow cytometer.

We observed that CD4⁺ and CD8⁺ T cells in MLR exhibit classical markers of response to allo- or xeno-antigenic stimulation. After 6 days of co-culture, human responder cells stimulated by porcine PBMCs displayed a higher proliferation of naive T lymphocytes and increased expression of CD25⁺ activation markers compared to stimulation with human PBMCs.

The data generated from MLR assays in both allogeneic and xenogeneic contexts support the development of a strategic immunomonitoring tool to assess the impact of genetic modifications in porcine cells, modulate the human immune response, with a particular focus on cellular immune mechanisms.

DECIT-CNPq #444097/2023-6, INCT-CNPQ-iii #408685/2024-7

Keywords: xenotransplantation;mixed lymphocyte reaction;cellular immunology.

3143 - Category: TRANSPLANTATION AND IMMUNOGENETICS (TR)

Investigation of XPC Gene Role in Inflammation Associated with Experimental Skin Transplantation

DE OLIVEIRA, T.G.¹; CIPELLI, M.²; CIONE, M.V.P.²; LEITE, J.A.³; DE OLIVEIRA, M.F.²; HIYANE, M.I.²; ALVES-SILVA, A.R.P.¹; CENEDEZE, M.A.¹; GIL, C.D.¹; CAMARA, N.O.S.²; SILVA FILHO, A.P.E.¹. 1. UNIFESP, SÃO PAULO - SP - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL; 3. UFRJ, SÃO PAULO - SP - BRASIL.

Introduction: The nucleotide excision repair (NER) system is essential for genomic stability, correcting DNA damage induced by ultraviolet radiation, oxidative stress, and carcinogenic agents. The XPC gene, responsible for the initial recognition of DNA lesions in the global-genome NER subpathway, has also been identified as a potential modulator of immunological processes. **Objective and Methods:** In this study, we evaluated the effect of XPC gene deficiency on the inflammatory response and graft survival in a murine model of skin transplantation. Wild-type and XPC knockout C57BL/6J mice received allogeneic skin transplants from BALB/c donors and were monitored for 14 days. **Results**: Graft survival, inflammatory infiltrate, histological changes, body weight, and tissue and serum levels of the pro-inflammatory cytokines TNF- α and IL-6 were analyzed at various time points after transplantation. XPC^{-/-} mice showed accelerated graft rejection, with early necrosis and greater inflammatory infiltrate in the initial days after the procedure. There was also a significant increase in TNF- α levels in XPC^{-/-} tissue on day 3 post-transplant, followed by a sharp decrease, indicating a dysregulated inflammatory response. **Conclusions:** Our preliminary results demonstrate that XPC gene absence significantly impacts allograft survival and inflammatory response dynamics. The accelerated rejection observed in XPC^{-/-} mice, combined with dysregulated cytokine production and enhanced early inflammatory infiltrate, suggests that DNA repair mechanisms play a crucial role in immune regulation during transplantation. These findings highlight the importance of genomic stability pathways in transplant outcomes and may contribute to the development of novel therapeutic approaches targeting DNA repair mechanisms to improve graft survival.

Keywords: DNA repair;XPC gene;skin transplantation.

2653 - Category: TUMOR IMMUNOLOGY (TU)

“Iron Oxide Nanoparticles Functionalized with an Approved Immunomodulator for Reprogramming Tumor-Associated Macrophages in Breast Cancer Therapy”

CARVALHO, J.G.; NASCIMENTO, C.S.; LAGE, A.C.P.; TAVARES, N.C.; MARQUES, T.M.; SANTOS, I.F.M.; ANTONELLI, L.R.D.V.; CALZAVARA-SILVA, C.E.. INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Breast cancer is the most prevalent and lethal type of cancer among women worldwide, highlighting the need for the development of new effective and safe therapeutic approaches. In the tumor microenvironment, tumor-associated macrophages (TAMs) play a key role in disease progression. Iron oxide nanoparticles (IONPs) have shown potential in reprogramming TAMs, promoting their polarization from an anti-inflammatory (M2) to a pro-inflammatory (M1) profile, thereby enhancing the antitumor immune response. This study evaluated the cytotoxicity of IONPs coated with a synthetic organic molecule (M11) and functionalized with an immunotherapeutic drug (Q1) in 4T1 tumor cells and murine macrophages. IONPs were characterized by DLS, FTIR, TEM, and endotoxin analysis. IONPs and IONPs-M11 had average diameters of 8.7 nm and 9.5 nm, respectively, and zeta potentials of -39.3 mV and -35.6 mV, indicating good colloidal stability. Q1 functionalization was efficient (92.84%), and all samples were endotoxin-free. MTT assays showed that IONPs-M11 (12.5; 25; 50; 100; 200 and 400 µg/mL) were non-cytotoxic to 4T1 cells and macrophages in any concentration, maintaining cell viability above 85%. Isolated Q1 (5, 10, 15 and 30 µg/mL) was also non-cytotoxic in any concentration to 4T1 cells, maintaining viability above 80%, however macrophage metabolic activity was increased up to 100%. IONPs-M11-Q1 (12.5; 25; 50; 100; 200 and 400 µg/mL) exhibited selective cytotoxicity toward tumor cells at concentrations above 25 µg/mL and increased macrophage metabolic activity up to 140% at 12 µg/mL. Concentrations of 12.5 and 25 µg/mL were selected for a further in vitro co-culture assay of 4T1 cells and macrophages to investigate the potential of IONPs-M11-Q1 as an immunotherapeutic strategy for TAM reprogramming and destruction of tumor cells. Financial support: FAPEMIG (RED-00079-22), Emendas Parlamentares (39600006), INOVA Fiocruz Terapias Avançadas (VPPIS-004-FIO-22).

Keywords: Breast cancer;Macrophages;Iron oxide nanoparticles.

2654 Category: TUMOR IMMUNOLOGY (TU)

“Cytotoxicity Evaluation of Functionalized Iron Oxide Nanoparticles with a TLR-7/8 Agonist in Breast Tumor Cells and Macrophages”

NASCIMENTO, C.S.; CARVALHO, J.G.; LAGE, A.C.P.; TAVARES, N.C.; MARQUES, T.M.; SANTOS, I.F.M.; ANTONELLI, L.R.D.V.; CALZAVARA-SILVA, C.E.. INSTITUTO RENÉ RACHOU-FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Breast cancer is the most prevalent and deadly type of cancer among women worldwide. Current treatment still faces significant challenges, which compromise patients' quality of life. In this context, the development of new therapeutic strategies capable of effectively and safely eliminating tumor cells is essential. In the tumor microenvironment, tumor-associated macrophages (TAMs) play central roles in tumorigenesis: M2-type TAMs, which are more abundant, promote tumor progression, while M1-type act to suppress neoplastic growth. Iron oxide nanoparticles (IONPs) have shown potential in TAM reprogramming, promoting the transition from an M2 to an M1 profile. This study evaluated the cytotoxicity of IONPs coated with a synthetic organic molecule (M11) and functionalized with an immunotherapeutic agent (Q2) in 4T1 tumor cells and murine macrophages. IONPs were characterized by DLS, FTIR, TEM, and endotoxin testing. IONPs and IONPs-M11 showed average diameters of 8.7 nm and 9.5 nm, respectively, and zeta potentials of -39.3 mV and -34.7 mV, indicating good colloidal stability. Functionalization with Q2 was efficient (89.58%), and all IONPs were endotoxin-free. MTT assays showed that IONPs-M11 was not cytotoxic to 4T1 cells and macrophages, maintaining cell viability above 85%. Isolated Q2 was also non-cytotoxic to 4T1 cells (100% viability) but increased macrophage metabolic activity by up to 100%. IONPs-M11-Q2 exhibited selective cytotoxicity toward tumor cells at concentrations above 100 µg/mL, while the maximum non-cytotoxic concentration for macrophages was 12.5 µg/mL. Thus, this concentration was selected for in vitro co-culture between 4T1 cells and macrophages, aiming to investigate the potential of IONPs-M11-Q2 as an immunotherapeutic strategy for TAM reprogramming and destruction of tumor cells. Financial support: FAPEMIG (RED-00079-22), Emendas Parlamentares (39600006), INOVA Fiocruz Terapias Avançadas (VPPIS-004-FIO-22).

Keywords: Breast cancer;Macrophages;Iron oxide nanoparticles.

2709 - Category: TUMOR IMMUNOLOGY (TU)

Effect of PBMC Incubation with Tumor Lysate on Lymphocyte Activation

TORRES, L.A.G.; COSTA, M.A.; SOUZA, I.D.F.; FREITAS, B.A.D.A.. UFVJM, DIAMANTINA - MG - BRASIL.

Triple-negative breast cancer (TNBC) is an aggressive form of the disease characterized by the absence of estrogen, progesterone, and HER2 receptors, which limits therapeutic options and makes chemotherapy the primary treatment approach. In the tumor pathophysiological context, an immunosuppressive tumor microenvironment is commonly observed, marked by the predominance of exhausted phenotype T lymphocytes, immunosuppressive cytokines, and M2-polarized macrophages, all contributing to tumor immune evasion. Strategies aimed at reversing this immunosuppressive profile and promoting leukocyte activation have been investigated as potential therapeutic adjuvants.

This study aimed to evaluate the ability of tumor lysate to promote lymphocyte activation from peripheral blood mononuclear cells (PBMCs) derived from healthy donors. The study was approved by the Ethics and Research Committee of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM), under approval number 7.548.477.

PBMCs were incubated for five days with lysate from MDA-MB-231 cells, a representative TNBC cell line, in 14-well plates containing RPMI medium supplemented with L-glutamine, fetal bovine serum, antibiotics, and antimycotics. After the incubation period, cells were stained with FITC-conjugated anti-CD28 antibody and analyzed by flow cytometry using a FACSCan system, with acquisition of 10,000 events within the lymphocyte gate.

Results showed an approximately 13-fold increase in the frequency of CD28⁺ cells in the samples stimulated with tumor lysate compared to the unstimulated controls. These findings suggest that tumor lysate may induce lymphocyte activation, representing a potential strategy to reverse the cellular exhaustion state observed in the tumor microenvironment. Further studies are necessary to assess whether this activation contributes to a more effective antitumor immune response.

Keywords: Tumor Lysate; Lymphocyte Activation; Triple-Negative Breast Cancer (TNBC).

2712 - Category: TUMOR IMMUNOLOGY (TU)

LYSIS OF TRIPLE-NEGATIVE BREAST CANCER CELLS MDA-MB-231 FOR IN VITRO LYMPHOCYTE STIMULATION

MOREIRA, M.S.. UFVJM, DIAMANTINA - MG - BRASIL.

Cancer is a complex disease that presents a major challenge to the global medical and scientific community. According to the National Cancer Institute (INCA), approximately 704,000 new cancer cases are expected annually in Brazil from 2023 to 2025. The tumor microenvironment in breast cancer is dynamic and intricate. T and B lymphocytes play a crucial role, with the ability to either promote or suppress tumor progression depending on the context. One strategy to enhance tumor suppression involves the pre-activation of immune cells—especially lymphocytes—so they can act more effectively within the tumor microenvironment.

This study aimed to establish a lysis protocol for MDA-MB-231 cells to subsequently stimulate lymphocytes in vitro. MDA cells were collected and centrifuged at 500 × g, at 18°C, for 10 minutes. The cell pellet was resuspended in 1000 µL of PBS. Cell counting was performed using a Neubauer chamber, and the concentration was adjusted to 1 × 10⁶ cells in 50 µL of PBS. Cells were subjected to five freeze-thaw cycles in PBS (−80°C to 37°C) on alternating days. This protocol effectively induced cell death. Qualitative analysis using trypan blue staining allowed for the identification of dead cells through microscopic observation.

Further studies are needed to determine the particle size in the lysate and assess its immunostimulatory potential. Although the lysis protocol effectively killed the cells, incomplete fragmentation suggests that protocol optimization is needed. Adjustments such as increasing the number of freeze-thaw cycles or introducing complementary techniques should be considered to enhance the release of tumor antigens and improve the efficacy of future experiments.

Keywords: Cancer immunotherapy; Tumor microenvironment; MDA-MB-231 cells.

2716 - Category: TUMOR IMMUNOLOGY (TU)

CD47 and CD24 as 'Don't Eat Me' Signals Associated with the Cancer Stem Cell (CSC) Phenotype in a Three-Dimensional (3D) Tumor Model as an Immune Evasion Mechanism in Human Cancers

GONÇALVES, B.Ô.P.; DA COSTA, L.M.P.; LOPES, L.M.S.. FUNDAÇÃO EZEQUIEL DIAS (FUNED), BELO HORIZONTE - MG - BRASIL.

One of the models that best explains the cellular heterogeneity in solid tumors is the presence of cancer stem cells (CSCs). In this context, three-dimensional tumor spheroids represent an *in vitro* model that replicates the organization and microenvironment of solid tumors, allowing the study of cancer growth, behavior, and drug responses in a more physiologically relevant context than 2D cultures. Tumor cells express surface molecules like CD47, CD24, and PD-L1, which act as "don't eat me" signals, helping them evade immune surveillance. Extracellular vesicles (EVs), including microvesicles and exosomes, play a key role in cell communication and may contribute to "don't eat me" signaling, promoting resistance to immunotherapy and sustaining cancer stemness. This study evaluated "don't eat me" signal expression in tumor spheroids generated from melanoma (SK-MEL-28) and non-small cell lung cancer (NCI-H226) cell lines. Cell subpopulations were analyzed by flow cytometry using CSC markers (CD24, CD44, CD133), mesenchymal markers (CD73, CD117, CD146), and the decoy death receptor TRAILR3. EVs were isolated from spheroid supernatants and characterized by Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), and flow cytometry. Spheroids morphology and cell distribution were assessed by confocal microscopy. Our results showed enrichment of CSC-like phenotype (CD44⁺/CD133⁺) in both models, with mesenchymal-like populations (CD73⁺/CD117⁺/CD146⁺) only in SK-MEL-28. NCI-H226 exhibited an epithelial profile (EGFR⁺/CD117⁻/CD146⁻). CD47 was detected in both spheroids. EVs expressed CD24, CD47, and EGFR and are signatures in both tumor models. These findings suggest that phenotypic characterization of "don't eat me" signals in spheroids tumors of melanoma and lung cancer may contribute to the development of improved therapeutic or diagnostic strategies.

Keywords: spheroids ;cancer;"don't eat me" .

2717 - Category: TUMOR IMMUNOLOGY (TU)

CRISPR/Cas9-mediated STAT3 knockdown for investigation of resistance mechanisms in tumor stem cell-enriched triple-negative breast cancer 3D spheroids (CD44⁺/CD24⁻/low).

ROCHA, B.1; MOREIRA, M.2; BARROS, B.A.F.1; GONÇALVES, B.Ô.P.2; CAMARGOS, B.N.C.1; LOPES, L.M.S.2. 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. FUNDAÇÃO EZEQUIEL DIAS, BELO HORIZONTE - MG - BRASIL.

Triple-negative breast cancer (TNBC) is aggressive, resistant to treatment and lacks defined therapeutic targets. This subtype is frequently enriched for CSCs with CD44⁺/CD24⁻ phenotype, associated with metastasis and resistance. STAT3 knockdown via CRISPR-Cas9 represents a promising approach to explore novel therapeutic targets in TNBC. In this study, 3D cultures of the TNBC cell lines MDA-MB-231 and HS 578T were used for analysis of cell viability, gene expression, cytometric phenotyping, confocal microscopy, in addition to the design of sgRNAs for STAT3 and CRISPR transfection. Doxorubicin (DOX) reduced spheroid viability at 180 µM (HS 578T) and 315 µM (MDA-MB-231), while salinomycin (SAL), known for its preferential cytotoxic action on tumor stem cells, showed efficacy at 9.68 µM and 14 µM, respectively. Confocal microscopy revealed that DOX treatment reduced the average size of spheroids of the MDA-MB-231 cell line, while salinomycin caused structural changes in the spheroids of both cell lines. Flow cytometry was used to evaluate the expression of CD44 and CD24 in TNBC cells after treatment. The results indicated that there were no significant changes in the CD44⁺/CD24⁻ phenotypic profile. In the gene expression assay, a significant increase in STAT3 was observed after DOX treatment, with an elevation of approximately 30x in MDA-MB-231 and 5x in HS 578T. The sgRNAs were designed using the Sigma, UCSC Genome Browser and CRISPOR platforms. In the MDA-MB-231 cell line, sgRNA1 did not induce detectable mutations, while sgRNA2 presented an intrinsic mutation in the region. In the HS 578T cell line, sgRNA2 showed evidence of the same mutation and was discarded. In contrast, sgRNA1 promoted detectable gene editing in this cell line. Complementary experiments are underway to confirm the efficiency of the editing. These findings reinforce the importance of the role of STAT3 as a possible therapeutic target in TNBC

Keywords: Triple-negative breast cancer;STAT3;Gene editing.

2738 - Category: TUMOR IMMUNOLOGY (TU)

Capsular Polysaccharides from *Cryptococcus neoformans* Suppress Cancer Through Apoptosis and Immune Cell Activation

LIMA, I.D.¹; DA FONSECA, L.M.¹; DE ALMEIDA, G.J.M.¹; DE OLIVEIRA, R.M.P.¹; DE SOUZA, M.E.R.¹; DOS SANTOS, I.M.F.²; DE OLIVEIRA, J.C.G.¹; MORROT, A.³; PREVIATO, J.O.¹; PREVIATO, L.M.¹; DECOTE-RICARDO, D.⁴; FREIRE-DE-LIMA, C.G.¹; DE LIMA, L.F.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 3. INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL; 4. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, SEROPÉDICA - RJ - BRASIL.

Cryptococcus neoformans is an opportunistic fungus that causes severe systemic infections in immunocompromised hosts, particularly in individuals weakened by cancer or chemotherapy. Its main virulence factors, the capsular polysaccharides glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal), modulate immune responses during infection and exhibit potential antitumor effects by inducing apoptosis and modulating cellular recruitment. To investigate this dual role, systemic *C. neoformans* infection was evaluated in murine breast cancer (4T1) and human lung cancer (A549) models. Although the infection worsened during tumor progression in mice, it unexpectedly reduced solid tumor growth while increasing ulcerations. Infected animals also displayed enhanced cytotoxic effector activity in the lungs and mammary tumors. Notably, tumors from infected mice showed higher PD-1 expression but reduced transcoelomic metastasis, suggesting infection simultaneously promotes immune exhaustion and restricts tumor dissemination. Mechanistically, purified GXM and GXMGal directly induced apoptosis in 4T1 and A549 cells *in vitro* while suppressing TGF- β production. Polysaccharide treatment also delayed 4T1 tumor progression *in vivo*, mirroring those effects observed during infection. Critically, these polysaccharides enhanced recruitment of cytotoxic cells and monocytes to tumor sites and upregulated CCR7 expression in both leukocytes and tumors. Collectively, these findings demonstrate that *C. neoformans* polysaccharides antagonize tumor growth through direct cytotoxicity and immune modulation. Although infection exacerbates immunosuppression, isolated GXM and GXMGal emerge as dual-function therapeutics, enhancing antitumor immunity and inhibiting metastasis, highlighting their potential as microbial-derived agents for cancer immunotherapy.

Keywords: *Cryptococcus neoformans*; capsular polysaccharides; antitumor activity.

2752 - Category: TUMOR IMMUNOLOGY (TU)

Effects of the combined inhibition of glutaminolysis and the STAT3 pathway on the tumor microenvironment and the PD-1/PD-L1 axis in Cervical Cancer

MARQUES, L.F.; LEPIQUE, A.P.. ICB-USP, SÃO PAULO - SP - BRASIL.

Background: One of the pathways tumor cells use to obtain energy and metabolic intermediates is glutaminolysis. There is evidence that inhibition of glutaminase, the enzyme that catalyzes the conversion of glutamine into glutamate in tumor-associated macrophages (TAMs), negatively impacts their tolerogenic phenotype. Signal Transducer and Activator of Transcription 3 (STAT3), a transcription factor upregulated in many tumors, promotes proliferation, immune evasion and glutamine metabolism. Our hypothesis is that blocking glutaminolysis in combination with STAT3 inhibition will reduce malignant cell viability and promote a cytotoxic phenotype in macrophages. We selected cervical cancer as our model because it relies on glutaminolysis and shows increased STAT3 activity. **Methods and Results:** Using 3D spheroid models from SiHa and SW756 cervical cancer cell lines, we found that glutaminase inhibitors (BPTES and compound 968) impaired tumor cell viability. In co-cultures with monocytes and tumor cells, treatment induced a mixed macrophage phenotype, increasing HLA-DR, CD80, CD206, and CD80⁺CD206⁺ cells. In treated cocultures with monocytes and tumor cells, lymphocytes displayed reduced suppression of CD4 T cell proliferation and increased CD69 expression. Cytokine analysis of culture supernatants showed that tumor cells caused a shift from cytotoxic cytokines to inflammatory and regulatory ones in lymphocytes. This effect was modulated by glutaminase inhibitors. When monocytes were added to cultures, there was downregulation of most cytokines induced by tumor cells, mainly when cultures were treated with glutaminase inhibitors. In triple population cultures, inhibitors increased IL-1 and IL-6 secretion, indicating a less suppressive environment. **Conclusion:** Our findings suggest that glutaminolysis supports cervical tumor cell survival and modulates the immune microenvironment by influencing macrophage phenotype, cytokine production, and T cell proliferation and activation.

Keywords: Cervical cancer; immunometabolism; glutaminolysis.

2755 - Category: TUMOR IMMUNOLOGY (TU)

Amphiregulin promotes immune suppression in colorectal cancer

SEVERINO, M.B.; DOS SANTOS, D.C.; DE MELO, B.M.S.; DAMASCENO, L.E.A.; LEANDRO, M.D.O.; FERNANDES, L.C.; CUNHA, T.M.; CUNHA, F.Q.; ALVES-FILHO, J.C.. USP, RIBEIRÃO PRETO - SP - BRASIL.

Colorectal cancer (CRC) is the third most common malignancy in Brazil and a leading cause of cancer-related mortality. CRC tumors are typically considered immunologically “cold,” characterized by low immune cell infiltration and a tolerogenic tumor microenvironment. Amphiregulin (AREG), a member of the epidermal growth factor (EGF) family, is notably overexpressed in colorectal tumors compared to other cancer types and is often associated with inflammation resolution. Given these features, this study aims to investigate the participation of AREG in orchestrating immune suppression in CRC. To this end, we first investigated the sources of AREG in the CRC microenvironment. Using single-cell analysis and bulk RNA sequencing of CRC biopsies, we identified dendritic cells (DCs) as an upregulated source of AREG in CRC. To explore its biological effects, we employed a CD11c-specific AREG-deficient mouse model (CD11c^{CRE}AREG^{fl/fl}). In vivo orthotopic administration of MC-38 colorectal cancer cells resulted in reduced growth, with smaller and lighter tumors in the absence of DC-derived AREG in the transgenic mice compared to those in wild-type mice. Ex vivo analysis of the tumors revealed a diminished population of INF γ ⁺ cytotoxic effector CD8⁺ T lymphocytes in the presence of AREG, besides a higher expression of immunosuppressive markers: CTLA-4, PD-L1, TIGIT, and LAG-3 compared to tumors from AREG-deficient DCs. Together, these findings demonstrate that DC-derived AREG impairs cytotoxic activity in CRC, highlighting its role in immune suppression and tumor progression. Therefore, targeting AREG may represent a promising therapeutic strategy to enhance antitumor immunity and improve patient outcomes.

Keywords: Colorectal cancer;Dendritic cell;Amphiregulin.

2761 - Category: TUMOR IMMUNOLOGY (TU)

Comparative Evaluation of rBCG-LTAK63 and Conventional BCG in Intravesical Immunotherapy Using an Optimized Orthotopic Bladder Cancer ModelROSA, B.G.¹; DE ALMEIDA, M.F.¹; GOZZI, I.C.C.¹; VLIET, J.C.V.¹; DELECHIAVE, D.²; LEITE, L.C.D.C.²; MORENO, A.C.R.². 1. UNIVERSIDADE DE SÃO PAULO - USP, SÃO PAULO - SP - BRASIL; 2. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

The development of relevant preclinical models is critical for advancing the study of intravesical immunotherapies for non-muscle invasive bladder cancer (NMIBC). In this study, we established a reproducible orthotopic murine model using MB49 urothelial carcinoma cells in C57BL/6 mice and evaluated the therapeutic potential of a recombinant BCG, rBCG-LTAK63, engineered to express the detoxified *Escherichia coli* heat-labile enterotoxin subunit LTAK63, a well-characterized mucosal adjuvant. Bladder tumor induction was achieved through urethral catheterization, epithelial disruption with 22% ethanol, followed by instillation of MB49 cells (1×10^3 or 5×10^3). Tumor establishment was assessed by hematuria and confirmed by bladder weight at day 22 post-instillation. The 5×10^3 cell dose exhibited consistent tumor implantation, validating the model's reproducibility and biological relevance for immunotherapy trials. To assess therapeutic efficacy, tumor-bearing mice received intravesical instillations of saline, BCG, or rBCG-LTAK63 on days 1, 8, 15, and 22. On day 25, bladders and spleens were collected for tumor burden analysis and multiparametric immune profiling by flow cytometry. Mice treated with rBCG-LTAK63 exhibited significantly improved control of bladder tumor volume compared to both BCG-treated and untreated groups. Immunologically, rBCG-LTAK63 treatment led to increased spleen frequencies of mature NK cells, dendritic cells (DC), and IFN- γ ⁺ CD8⁺ T cells, concomitant with reduced regulatory T cell (Treg) populations. Within the tumor microenvironment rBCG-LTAK63 induced greater infiltration of DCs and macrophages and a decrease in Treg, suggesting a reshaped, immunostimulatory environment. Altogether, we established a reliable orthotopic model for bladder cancer in mice and provided first evidence that rBCG-LTAK63 induces a superior antitumor potential than conventional BCG. These results reinforce the translational promise of rBCG-LTAK63 for NMIBC treatment.

Keywords: Bladder Cancer;Orthotopic Model;Intravesical Immunotherapy.

2763 - Category: TUMOR IMMUNOLOGY (TU)

Use of low doses of Cyclophosphamide and 5-Fluorouracil in melanoma mouse model

DE PAIVA, K.L.R.; SIMÕES, M.M.; FARIAS, G.R.; DE SOUZA, I.F.; MELO, V.C.; FONSECA, M.J.P.; MUEHLMANN, L.A.; BAO, S.N.. UNIVERSIDADE DE BRASILIA, BRASILIA - DF - BRASIL.

Introduction: Melanoma is one of the most immunogenic cancers, making it an excellent model for studying the complex interactions between tumor cells and the host immune system. Immunosuppressive cell populations, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), contribute to tumor immune evasion and resistance to immunotherapy. Cyclophosphamide (CY) and 5-fluorouracil (5-FU) have been shown to selectively deplete these populations. However, the broader molecular and immunological consequences of low-dose chemotherapy on the tumor microenvironment remain incompletely understood.

Objective: This study aimed to investigate the immunomodulatory effects of low-dose cyclophosphamide and 5-fluorouracil in a B16-F10 melanoma mouse model, with a focus on changes in tumor progression, survival, and gene expression related to immune regulation. **Methods:** C57BL/6J mouse model, were divided into control groups and treated with low doses of cyclophosphamide (CY) and 5-fluorouracil (5-FU). We monitored tumor growth and overall survival. Additionally, bulk mRNA sequencing of tumor tissues was performed to assess changes in gene expression profiles following treatment.

Results: The combination of low-dose CY and 5-FU significantly delayed tumor progression and improved overall survival compared to untreated controls. Transcriptomic analysis revealed a notable downregulation of genes associated with immune activation pathways, suggesting suppression of immune pathways in the tumor microenvironment. **Conclusion:** This study highlights the complex immunological impact of low-dose chemotherapy in melanoma. While the combination of CY and 5-FU delayed tumor growth and extended survival, transcriptomic data revealed suppression of immune-related gene expression, suggesting an immunoregulatory shift in the tumor microenvironment. These findings provide a foundation for designing more effective combinatorial treatment strategies.

Keywords: melanoma;;low-dose chemotherapy;tumor microenviroment.

2764 - Category: TUMOR IMMUNOLOGY (TU)

Photodynamic Therapy with Aluminum Phthalocyanine Nanoparticles Induces Immunogenic Cell Death and Activates Antigen-Presenting Cells in Murine Melanoma In Vitro

SIMÕES, M.M.; DE PAIVA, K.L.R.; DE SOUZA, I.F.; MELO, V.C.; BAO, S.N.. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

Photodynamic therapy (PDT) generates reactive oxygen species, inducing oxidative stress that triggers immunogenic cell death (ICD) via the release of damage-associated molecular patterns (DAMPs) [1]. These molecules act as immunostimulatory signals that promote the activation of antigen-presenting cells (APCs). Their recognition by APCs is essential for adaptive immune activation, especially of CD8⁺ T cells, which are central in tumor control. PDT is thus a promising non-invasive therapy due to its ability to stimulate antitumor immunity [2]. However, its clinical use is limited by poor photosensitizer bioavailability and low photostability [3]. To overcome this, the group developed a third-generation photosensitizer: aluminum phthalocyanine chloride in solid lipid nanoparticles from Amazonian butter (AlPcNLS), aimed at enhancing pharmacokinetics, photodynamic activity, and immunomodulation [4]. The aim of this study was to evaluate the potential of PDT with AlPcNLS to induce immunogenic cell death and activate antigen-presenting cells. B16-F10 melanoma cells and APCs derived from C57BL/6 mouse bone marrow were used. Tumor cells were treated with AlPcNLS for 15 minutes, followed by incubation in the dark or LED irradiation (660 nm, 25.88 J/cm², 10 min). APC activation was assessed by flow cytometry, and cytokine levels were measured by ELISA. Immunostaining for confocal microscopy and transmission electron microscopy showed that the treatment induced the production of DAMPs. APCs exhibited increased expression of membrane components such as MHC II, CD80, and CD86, as well as elevated production of cytokines such as IL-12 and IFN- γ , which are key factors for immune system activation. These results indicate that the established protocol using the developed nanostructure has the potential to enhance PDT efficacy by promoting immunogenic cell death and inducing phenotypic changes in APCs indicative of their activation.

Keywords: photodynamic therapy;immunogenic cell death;antigen-presenting cells.

2765 - Category: TUMOR IMMUNOLOGY (TU)

Investigating the effect of Butyl-Coumarate on CD1d Expression in Melanoma

DE QUEIROZ, A.M.; LABATE, J.D.L.; KELLER, A.C.. UNIFESP, SÃO PAULO - SP - BRASIL.

The invariant natural killer T (iNKT) cells represent a non-conventional subset of T lymphocytes that recognize glycolipid antigens presented by the CD1d molecule. iNKT cell activation with specific agonists, such as α -galactosylceramide (α GC), induces both direct and indirect cytotoxicity against tumors, and enhances the cytotoxic activity of T lymphocytes and NK cells. Nonetheless, the low expression of CD1d in transformed cells limits the effectiveness of iNKT-based immunotherapy, especially in solid tumors. Several epigenetic mechanisms modulate CD1d expression, including the activity of histone deacetylase (HDAC). Considering that coumaric acid (*p*-Ca) has a potent HDAC inhibitory activity and that its esterification improves several biological activities, we aimed to investigate the effect of its ester derivative, butyl-coumarate (*b*-*p*Ca), on CD1d expression in melanoma cells. To evaluate CD1d expression, B16-F0 and B16-F10 melanoma cells were treated with *b*-*p*Ca for 24 hours. We found that *b*-*p*Ca treatment increased both CD1d mRNA transcription and cell surface protein expression. In addition, *b*-*p*Ca treatment enhanced the response of iNKT cells to B16 cells loaded with α GC. Further analysis demonstrated that *b*-*p*Ca did not reduce the transcript levels of HDAC1 and HDAC2, nor increase acetylated histone H3 levels, indicating that its effect on CD1d expression is independent of classical HDAC inhibition. In conclusion, our findings identify *b*-*p*Ca as a promising alternative for upregulating CD1d expression in melanoma cells through a mechanism that does not involve histone acetylation.

Keywords: CD1d;*b*-*p*Ca;iNKT-based immunotherapy.

2770 - Category: TUMOR IMMUNOLOGY (TU)

Advanced approaches to investigate Tumor Infiltrating Lymphocytes (TILs) and their function

MOURA, L.V.¹; AMARANTE-MENDES, G.P.¹; OHASHI, P.². 1. USP, SÃO PAULO - SP - BRASIL; 2. UNIVERSITY HEALTH NETWORK, TORONTO - CANADA.

Tumor-infiltrating lymphocytes (TILs) are T cells that recognize tumor antigens and naturally migrate into the tumor microenvironment. Their use in adoptive cell therapy has shown promising results, particularly in refractory solid tumors. TIL expansion occurs in two distinct phases: the Pre-Rapid Expansion Protocol (Pre-REP) and the Rapid Expansion Protocol (REP). This study focused on the Pre-REP phase, during which TILs were cultured for 30 days in a medium containing high concentrations of interleukin-2 (IL-2). Seven tumor samples were analyzed (six ovarian cancers and one melanoma), categorized based on proliferative capacity as fast growers (>30 million cells) or slow growers (<30 million). After culture, cells were evaluated by flow cytometry using two antibody panels: one focused on immunophenotyping and the other on exhaustion markers. The analyses identified various immune populations (CD4⁺, CD8⁺, Tregs, NK, B cells, among others), as well as the expression of activation markers (CD69, 4-1BB, HLA-DR) and exhaustion markers (PD-1, TOX, CTLA-4, TIM-3) at both day 0 and day 30. Expected changes were observed over time, but no statistically significant differences were found between growth groups, except in the frequency of NK cells, which differed between fast and slow growers. Subanalysis of the CD56⁺ CD16⁻ NK subset, associated with immunoregulatory function, showed no significant variation. Proliferation (Ki67) and effector function (Granzyme B) were also evaluated. UMAP and clustering analyses revealed two distinct clusters, with statistical difference only within the fast-growing group between days 0 and 30. In conclusion, although phenotypic differences were observed, no robust predictive markers of TIL expansion were identified. Further studies are needed to better understand the determinants of successful expansion and to optimize the therapeutic application of TILs.

Keywords: Tumor Infiltrating Lymphocytes;Spectral Flow Cytometry;Exhaustion.

2771 - Category: TUMOR IMMUNOLOGY (TU)

Combination of rBCG-S1PT and IL-15 Superagonist Promotes Central Memory T Cell Expansion and Tumor Control in Murine Model of Bladder Cancer

GOZZI, I.C.C.; ROSA, B.G.; VLIET, J.C.V.; DE ALMEIDA, M.F.; LEITE, L.C.D.C.; MORENO, A.C.R.. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

Non-muscle invasive bladder cancer (NMIBC) accounts for more than 70% of bladder cancer cases. In this context, the standard treatment consists of transurethral resection of the tumor followed by cycles of immunotherapy with Bacillus Calmette-Guérin (BCG). However, many patients are unresponsive to this treatment, making it necessary to seek more effective therapies. In the Vaccine Development Laboratory, a recombinant strain of BCG (rBCG) was developed, which expresses the S1 subunit of detoxified pertussis toxin (rBCG-S1PT). Recently, immunotherapy with IL-15 superagonists (SA IL-15) has demonstrated great therapeutic efficacy in the treatment of NMIBC in clinical trials. Based on this, the present work aimed to investigate the antitumor effects of BCG or rBCG S1PT in association with SA IL-15 in a murine model of orthotopic bladder cancer. After four cycles of immunotherapy with BCG or rBCG associated with SA IL-15 inoculation and twenty five days after tumor implantation, animals were euthanized, organs were collected and the cellular immune response was analyzed by cytometry. The association between SA-IL-15 and BCG or rBCG showed greater antitumor potential than monotherapies. In addition, an increased infiltration of CD4 T and NKT cells on tumor, which exert antitumor activity, was observed in the groups treated with the combination therapies. The combination of rBCG + SA IL-15 promoted an increase in central memory CD8 T cells in the spleen, indicative of sustained immune surveillance and serving as key biomarkers of effective immunotherapies. Furthermore, the combined therapies reduced the toxicity associated with super agonist monotherapy, attenuating signs of hepatosplenomegaly and hydronephrosis. Taken together, these findings highlight the therapeutic potential of combining rBCG-S1PT with SA IL-15 as a novel immunotherapeutic strategy for NMIBC, positioning it as a promising candidate for future translational studies.

Keywords: Bladder cancer;Recombinant BCG;Combination therapy.

2774 - Category: TUMOR IMMUNOLOGY (TU)

Effect of STAT3 Inhibition Combined with Conventional Therapies in Ovarian Cancer

BUTARELLI, A.L.D.A.¹; DIZ, M.D.P.E.²; LEPIQUE, A.P.¹. 1. UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. SÃO PAULO STATE CANCER INSTITUTE, SÃO PAULO - SP - BRASIL.

Ovarian cancer (OC) is among the most common and lethal gynecological malignancies worldwide. High-grade serous carcinoma is the most prevalent subtype and the main contributor to OC-related mortality. Although OC has a multifactorial etiology, about 10–15% of cases are linked to mutations in DNA repair genes such as BRCA1 and BRCA2. In these tumors, cancer cells rely on PARP (poly ADP-ribose polymerase) activity as a resistance mechanism to chemotherapy. Current treatments include surgical resection combined with chemo- or radiotherapy, along with angiogenesis and PARP inhibitors. However, recurrence and resistance rates remain high. The OC tumor microenvironment consists of malignant cells, fibroblasts, endothelial cells, and immune infiltrates, and often shows constitutive activation of STAT3, a signaling molecule with oncogenic and immunosuppressive roles. Based on this, we hypothesize that inhibiting STAT3—combined with PARP inhibition (in BRCA1/2-deficient tumors) or chemotherapy (in BRCA-proficient tumors)—may enhance tumor damage and boost antitumor immune responses. In the PARP inhibition context, activation of the cGAS/STING pathway may act synergistically with STAT3 blockade to promote cytotoxic immunity. Preliminary data from our group showed that dual inhibition of STAT3 and PARP in the OC cell line OVCAR-3 had additive effects on G2/M cell cycle arrest and induction of cell death. Our next step involves testing this therapeutic strategy in ex vivo 3D models of the tumor microenvironment, established from ascites-derived tumor cells. In these models, we will evaluate treatment effects on tumor viability and immune cell activation. Based on our data and current literature, we expect increased tumor cell death and activation of cytotoxic immune responses. This study is funded by the São Paulo Research Foundation (FAPESP), grants #2022/10288-5 and #2024/17550-8; and CNPq grant #310154/2021-9.

Keywords: Antitumor immune response;Combination therapy;Ex vivo tumor models.

2784 - Category: TUMOR IMMUNOLOGY (TU)

Downregulation of Enteroendocrine Genes Predicts Survival in Colon Cancer: A Bioinformatics-Based Analysis

DA SILVA, E.M.¹; CIPELLI, M.²; AMARAL, M.A.²; SILVA FILHO, A.P.E.¹; CAMARA, N.O.S.²; ANDRADE-OLIVEIRA, V.³.
1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. FEDERAL UNIVERSITY OF ABC, SÃO PAULO - SP - BRASIL.

Colorectal cancer (CRC) is the fourth most common cancer and the third most deadly worldwide. Even with alternative therapies, some patients do not respond to treatment. Identifying modulations in the tumor microenvironment (TME) of CRC is indeed a challenge due to the complex and dynamic nature of the TME. Enteroendocrine cells (EECs) are a secretory-lineage intestinal epithelial cell type that produces more than 20 hormones, making them a key component of the body's endocrine system. Among these hormones, the group of incretins (GIP and GLP-1) stands out, which influence the modulation of macrophages and lymphocytes in an inflammatory context, in addition to their agonists functioning as a regulator of intestinal tumorigenesis. However, the relevance of EECs in the TME is still a matter of debate. Some studies suggest their involvement in tumor development, while others indicate a protective role, depending on the type of cancer. This study investigated the involvement of markers of EECs and their hormones in CRC through an integrative bioinformatics analysis using publicly available datasets from NCBI and TCGA, encompassing both human and mouse CRC samples. Our findings reveal a TME characterized by elevated WNT pathway activity and increased expression of Paneth cell-associated markers and transcription factors, such as WISP1, also known as CCN4 (Cellular Communication Network Factor 4), LYZ (Lysozyme), SOX9 (SRY-Box Transcription Factor 9), and DEFA1 (Defensin alpha 1). Strikingly, CRC tissue exhibited a significant downregulation of hormone secretion pathways and reduced expression of enteroendocrine-specific gene markers compared to healthy tissues. Notably, lower levels of GCG (Glucagon – that encoding GLP-1) and CHGA (Chromogranin A) correlated with reduced overall survival and chemotherapy-responsive patients. These results suggest that disruption of enteroendocrine signaling is a hallmark of CRC development and may hold prognostic and therapeutic value.

Keywords: colorectal cancer;enteroendocrine;immune cells.

2788 - Category: TUMOR IMMUNOLOGY (TU)

Investigation of dietary influence on lipid droplets in colon cancer development in vivo

BRANCO, J.M.C.R.; VIOLA, J.P.D.B.; VIEIRA, B.L.M.D.O.. INSTITUTO NACIONAL DE CANCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

Colon cancers are highly prevalent worldwide and are associated with obesity and inflammation, strongly influenced by individual habits, especially diet. Advances in food processing have increased the consumption of hypercaloric foods, leading to elevated caloric intake and lipid accumulation in cells, which interferes with metabolic pathways linked to poor tumor prognosis. In cancer, altered lipid metabolism involves metabolic regulators and may offer therapeutic targets. This study investigates the influence of dietary patterns on the profile and frequency of lipid droplets (LDs) in tumor progression using a murine model. C57BL/6J mice were subjected to colorectal carcinogenesis with azoxymethane (AOM) and dextran sulfate sodium (DSS), followed by three diets: standard chow (control), high-fat and high-sucrose (HFHS), and a fast-food mimicking diet (FFMD). Mice fed HFHS and FFMD showed impaired glucose tolerance (ipGTT), indicating diet-induced insulin resistance. Hepatic steatosis and splenomegaly were also observed. Thirty days after DSS treatment, both tumor number and size increased in HFHS and FFMD groups. Mesenteric lymph node analysis showed no changes in B cells or CD4+/CD8+ T cell populations. CD4+ T cell activation remained unchanged, but CD25 expression decreased on CD8+ T cells, possibly reflecting increased activation. These CD8+ T cells showed elevated exhaustion markers (PD-1, PD-L1), especially in hypercaloric diet groups. IFN- γ production was also reduced in CD8+ T cells, likely due to exhaustion. Ongoing analyses aim to further characterize the inflammatory tumor microenvironment and the role of LDs in modulating tumor progression.

Keywords: Lipid Droplets;Diet-induced carcinogenesis;Colon cancer.

2791 - Category: TUMOR IMMUNOLOGY (TU)

Next-Generation BCG Immunotherapy for NMIBC: Preclinical Efficacy and Human Translational Insights

DE ALMEIDA, M.F.¹; DELECHIAVE, D.²; ROSA, B.G.³; VLIET, J.C.V.¹; GOZZI, I.C.C.¹; TRENTINI, M.M.²; LEITE, L.C.D.C.²; MORENO, A.C.R.². 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Immunotherapy with *Bacillus Calmette-Guérin* (BCG) remains the gold standard for the treatment of non-muscle invasive bladder cancer (NMIBC). However, therapeutic resistance and relapse remain significant challenges, underscoring a critical gap in the efficacy of current treatments. In this context, our group developed the recombinant strain rBCG-LTAK63, engineered to exhibit enhanced adjuvant properties and elicit a more robust antitumor immune response. This study aimed to evaluate and compare the immunomodulatory profile and antitumor efficacy of rBCG-LTAK63 with the parental BCG strain. Using a heterotopic murine model of bladder cancer, we observed that immunotherapy with rBCG-LTAK63 resulted in significantly improved tumor control compared to BCG. This enhanced efficacy was associated with a stronger immune response, both systemically and within the tumor microenvironment. Treatment with rBCG-LTAK63 led to increased activation of dendritic cells and CD8⁺ T lymphocytes, along with a reduction in local immunosuppression through decreased frequencies of FoxP3⁺ regulatory T cells. Additionally, we explored the translational potential of rBCG-LTAK63 by assessing its ability to activate human monocyte-derived macrophages. We observed that stimulation with rBCG-LTAK63 resulted in a significantly stronger macrophage activation phenotype, marked by upregulated TNF production, indicating superior immunostimulatory capacity relative to BCG. These data collectively underscore the translational potential of rBCG-LTAK63 as a next-generation immunotherapeutic strategy for NMIBC, capable of eliciting a potent immune response and addressing key limitations of current BCG-based treatments.

Keywords: Bladder Cancer;Immunotherapy;Translational Research.

2793 - Category: TUMOR IMMUNOLOGY (TU)

Combining Recombinant BCG and Low-Dose Anti-PD-1: A Potent and Affordable Strategy for NMIBC Treatment

VLIET, J.C.V.¹; DE ALMEIDA, M.F.¹; GOZZI, I.C.C.¹; ROSA, B.G.¹; FERREIRA, L.C.D.S.¹; LEITE, L.C.D.C.²; MORENO, A.C.R.². 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

Non-muscle invasive bladder cancer (NMIBC), the most common form of bladder cancer, is treated with intravesical BCG. However, supply shortages and frequent treatment failure highlight the need for alternatives. Although anti-PD-1 therapies are approved for BCG-unresponsive cases, their cost and toxicity limit use. To address this, we developed rBCG-LTAK63, a recombinant BCG expressing the A subunit of *E. coli* heat-labile enterotoxin, which exhibits enhanced antitumor activity. Here, we investigated the therapeutic potential of combining BCG or rBCG with anti-PD-1 in a murine model, focusing on anti-PD-1 dose optimization. *In vitro*, MB49-GFP bladder cancer cells were co-cultured with murine splenocytes, infected with BCG or rBCG, and treated with anti-PD-1 after 6 hours. Fluorescence (as a proxy for cell viability) and cytokine production were assessed. Anti-PD-1 alone had no effect, while BCG reduced fluorescence. The combination enhanced tumor cell killing, particularly with rBCG. Flow cytometry showed that anti-PD-1 reduced TNF- α in CD4⁺ T cells, while BCG and rBCG increased it. Only the rBCG + anti-PD-1 combo restored TNF- α levels, indicating a distinct immunomodulatory effect. *In vivo*, anti-PD-1 (50 or 100 μ g) was given intraperitoneally on days 8, 11, and 14 after orthotopic tumor implantation (5×10^3 MB49 cells) in C57BL/6 mice. Notably, the 100 μ g dose, half the amount typically used, still reduced bladder tumor weight, despite mild increases in urea and platelets. In survival assays with lower tumor burden (2×10^3 cells), mice received intravesical BCG or rBCG (10^5 CFU on days 1, 8, 15, 22) and anti-PD-1 (100 μ g on days 8, 11, 14). Combination therapy improved survival over monotherapies, with the rBCG + anti-PD-1 group showing the best outcome. These results highlight this strategy as a potent, scalable, and more affordable option for NMIBC, especially in resource-limited settings.

Keywords: bladder cancer ;recombinant BCG;immune-checkpoints

2819 - Category: TUMOR IMMUNOLOGY (TU)

IL-18 expressed by *Salmonella* Typhimurium can modulate regulatory pathways in response to proliferation of aggressive and metastatic colorectal cancer organoids in vitro.

QUIARIM, T.M.¹; FRAGELLI, B.D.D.L.¹; DA SILVA, A.J.¹; ANIBAL, F.¹; KESSELRING, R.². 1. UFSCAR, SÃO CARLOS - SP - BRASIL; 2. UNIVERSITÄTSKLINIKUM FREIBURG, FREIBURG IM BREISGAU - ALEMANHA.

Colorectal cancer (CRC) is the third most prevalent cancer in the world and the second leading cause of cancer-related deaths. Even with considerable progress in cancer therapy, several patients face challenges due to chemotherapy resistance, highlighting the need for new treatment approaches, especially in metastatic cases. Genetically engineered *Salmonella* Typhimurium has emerged as a promising immunotherapeutic vector capable of targeting tumors, delivering antitumor agents, modulating the tumor microenvironment, and stimulating antitumor immunity. Interleukin-18 (IL-18), a pro-inflammatory cytokine involved in mucosal homeostasis, has shown potential in inhibiting CRC proliferation, making it a compelling candidate for antitumor therapy. This study investigated the effects of pro-IL-18 and bioactive IL-18, expressed by *Salmonella* Typhimurium, SL3261, on aggressive CRC organoids *in vitro*. IL-18 gene was cloned into plasmid pAE, containing nirB promoter, and inserted into SL3261, with protein expression induced under anaerobic conditions. Western blot analysis of cytoplasmic and nuclear extracts were performed on APTAK organoids, derived from mouse tumor of intestinal epithelial cells lacking APC, TP53, Tgfr2, and expressing KRASG12D and act/myr-Akt1. Organoids derived from primary CRC tumors, liver metastases, and peritoneal carcinomatosis (from APTAK-transplanted mice) were also evaluated. IL-18 suppressed β -catenin and GSK-3 β activation and altered STAT1 and STAT3 signalling, with nuclear STAT3 stimulation particularly evident in primary tumor and metastatic cells. p38 MAPK was suppressed in the nucleus but increased in the cytoplasm with bioactive IL-18. NF κ B was suppressed in the cytoplasm and mildly activated in the nucleus. These findings suggest that both forms of IL-18, expressed by SL3261, can modulate regulatory pathways associated with proliferation, inflammation and tumor cell survival, supporting its potential as a candidate for CRC immunotherapy.

Keywords: CRC immunotherapy; *Salmonella* Typhimurium SL3261; Interleukin-18 (IL-18).

2821 - Category: TUMOR IMMUNOLOGY (TU)

A low-cost reagent for lentiviral vector concentration to improve accessibility of gene therapies

SCOTTON, C.V.¹; PIVETTA, R.S.²; DE SOUZA, L.L.²; SUAREZ, E.R.². 1. UNIVERSIDADE FEDERAL DO ABC, SÃO BERNARDO DO CAMPO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, SANTO ANDRÉ - SP - BRASIL.

Cellular gene therapy using T cells engineered to express chimeric antigen receptors (CARs) targeting tumor-associated antigens has shown promising results in the treatment of some hematological cancers. However, the high costs associated with producing this therapy limit its use. Lentiviruses are the most widely used vectors for CAR T cells manufacturing.

This project aims to enhance the efficiency of a reagent used for lentiviral vector concentration by testing polyethylene glycol (PEG) with variable molecular weights, varying albumin concentrations, and different centrifugation volumes, compared to the high-cost gold standard reagent for lentiviral concentration.

Plasmid vectors were amplified and purified, and lentiviruses were produced via transient transfection of five plasmids into HEK293T cells using polyethylenimine (PEI). Viral particles were then concentrated under various conditions, including different PEG molecular weights and albumin concentrations. Centrifugation was performed using varying volumes in a Sorvall bottle. The transduction levels were assessed using the same volume of viral supernatant. The viral titer and respective efficiency of the optimized reagent were evaluated by flow cytometry.

Among the PEG variants tested, PEG 6000 without albumin provided the best balance between titer and yield, very similar to the high-cost gold standard reagent for lentiviral concentration.

Our findings will contribute to the development of a more cost-effective gene therapy, based on nationally produced reagents, making lentiviral-based gene therapies more accessible for the population.

Approved by: CEP/UFABC CAAE 71055617.3.0000.5594; CIBio/UFABC CQB 0304/10 001/2017; Supported by: FAPESP n°2023/03631-3 and PIC/UFABC.

Keywords: Cost; T cells; Lentivirus.

2825 - Category: TUMOR IMMUNOLOGY (TU)

Estrogen suppression-induced osteoporosis fosters bone metastases in EO771B luminal B breast cancer model by modulating pro-tumoral immune responses

GUERREIRO, M.A.D.S.¹; GARCIA, D.D.A.²; MASSET, M.E.G.²; ALVES, I.N.D.S.²; XAVIER, A.M.¹; SANTO, K.P.D.S.¹; SANTOS, C.S.P.D.O.¹; CANTO, F.¹; MONTEIRO, A.C.¹. 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Breast cancer is the most prevalent malignant tumor among women, with approximately 70% of patients developing bone metastases—an event associated with poor prognosis, particularly in those with the luminal B subtype characterized by positive hormone receptor expression (ER⁺/PR⁺). These metastases arise from dysregulation of bone remodeling, a process involving skeletal cells and the RANK/RANKL/OPG axis, and are marked by exacerbated osteoclast activity, often intensified by estrogen deficiency and modulated by immune cells. While estrogen deficiency is known to contribute to bone loss and disruption of the skeletal microenvironment, it remains unclear whether osteoporosis increases susceptibility to bone metastasis. To address this, we investigated whether osteoporosis induced by estrogen suppression prior to tumor onset functions as a risk factor for bone metastasis, as well as its impact on the modulation of pro-tumoral immune responses. Osteoporosis was induced with leuprolide over six weeks, after which animals were orthotopically inoculated with EO771B cells, a murine luminal B breast cancer line. After 32 days, mice were euthanized and organs were collected. Bone metastases were assessed through metastatic clonogenic assay, and immune profiling was performed by flow cytometry. A marked reduction in reproductive organ weight confirmed estrogen suppression. Notably, leuprolide-treated animals exhibited increased tumor colonies in iliac bones and vertebrae. These findings suggest that osteoporosis may represent a risk factor for bone metastasis, potentially by modulating pro-tumoral immune responses.

Keywords: Breast Cancer;Osteoporosis;Bone Metastasis.

2830 - Category: TUMOR IMMUNOLOGY (TU)

Commensal microbiota-driven modulation of RANKL⁺ T cells and its impact on tumor-bone crosstalk in the 4T1 mouse mammary carcinoma

MASSET, M.E.G.¹; GARCIA, D.D.A.¹; ALVES, I.N.D.S.¹; SANTOS, C.S.P.D.O.²; SANTO, K.P.D.S.²; GUERREIRO, M.A.D.S.²; XAVIER, A.M.²; DOS SANTOS, H.F.²; CANTO, F.²; MONTEIRO, A.C.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL.

Our group has previously reported that, in response to the 4T1 murine breast carcinoma (4T1 BC), RANKL-producing T cells infiltrate the bone marrow (BM) prior to tumor cell arrival, establishing an early osteolytic disease in a RANKL-dependent manner that primes the BM for metastatic colonization. Recently, significant advances have been made in elucidating the role of the commensal microbiota in bone remodeling dynamics, particularly through T cell regulation, thereby defining the bone-gut axis. To investigate the involvement of this axis in the T cell-dependent progression of 4T1 BC, we employed oral administration of a broad-spectrum antibiotic cocktail (ampicillin, neomycin, metronidazole, and vancomycin [VMNA]) to deplete the microbiota. 4T1 BC-bearing mice treated with VMNA (4T1 VMNA) exhibited fewer metastatic clones in both tumor-draining lymph nodes (dLN) and the BM. In vitro, BM cells from 4T1 VMNA mice displayed a secretory profile less capable of promoting osteoclast differentiation compared to cells from untreated 4T1-bearing mice. Consistently, micro-CT-based morphometric analysis of iliac bone showed that microbiota depletion attenuated 4T1-associated bone loss. Flow cytometric profiling of T cells in the dLN revealed that microbiota depletion increased the frequency of RANKL⁺CD4⁺ T cells, particularly within the Foxp3⁻ conventional CD4⁺ T cell subset. However, the absolute number of these cells was reduced compared to untreated 4T1-bearing mice, likely reflecting overall changes in cellularity or T cell recruitment. In the mesenteric LN, 4T1-bearing mice exhibited a higher frequency of CD4⁺RANKL⁺T cells, regardless of VMNA treatment, when compared to tumor-free controls. Collectively, our data suggest that the commensal microbiota acts as a deleterious factor in 4T1 BC-induced bone disease and highlight novel avenues for exploring the influence of microbial communities on the RANK-RANKL axis and the pathogenic potential of RANKL⁺T cells.

Keywords: breast cancer;T cells;Bone.

2842 - Category: TUMOR IMMUNOLOGY (TU)

Immune Exhaustion Signatures and Biomarker Correlates of Treatment Response in Stage III Melanoma Treated with Adjuvant Anti-PD-1.

APOSTOLICO, J.D.S.¹; RABELLO, M.I.D.S.¹; FIGUEIREDO, A.B.¹; EVANGELISTA, G.F.D.B.¹; MORAIS, K.L.P.¹; FERREIRA, S.M.I.¹; KUIL, L.D.M.²; DE LIMA, V.C.C.³; SILVA, M.J.D.B.E.³; GOLLOB, K.J.¹. 1. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. A.C.CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 3. CLINICAL ONCOLOGY DEPARTMENT, A.C.CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

Immunotherapy with immune checkpoint inhibitors has revolutionized cancer treatment in the last decade and has demonstrated promising results in treating various types of tumors. The use of anti-PD-1 antibodies has emerged as an interesting approach in the adjuvant treatment of patients with melanoma, demonstrating success in preventing recurrence and metastasis of the disease in some patients. However, 30% of patients who receive this type of treatment experience disease recurrence within the first year, in addition to distant metastasis and tumor recurrence after the end of treatment. We evaluated the immunological mechanisms associated with response to therapy and tumor progression in patients with primary melanoma undergoing adjuvant treatment with immune checkpoint inhibitors. In a prospective cohort of 30 patients, whole blood samples were collected to analyze the soluble and cellular immune profiles, including plasma levels of 32 soluble immune mediators by multiplex platform and profile immune cell by flow cytometry. The results revealed distinct differences in immune subpopulations between the R and NR groups. Notably, there is a negative correlation between PD1+ and CTLA4+ T cell subpopulations, markers of immune exhaustion, and several inflammatory and anti-inflammatory cytokines tested in NR group. This correlation profile differs between R and NR patient groups, suggesting a potential role in treatment response. These findings highlight the need for further studies in this cohort to elucidate the mechanisms driving immune exhaustion and its impact on therapeutic outcomes. Future analyses may provide valuable insights into novel therapeutic targets and enhance patient stratification strategies, paving the way for more personalized treatment approaches. This study was approved by the Research Ethics Committee of the Hospital Israelita Albert Einstein (CAAE: 54281321.6.0000.0071; #6.821.782).

Keywords: melanoma;adjuvant treatment;anti-PD-1.

2855 - Category: TUMOR IMMUNOLOGY (TU)

CD163 knock-out in human monocyte-derived macrophages by CRISPR-Cas9 using a lentiviral platform: First perspectives

FOGOLIN, C.S.¹; PEREIRA, V.A.C.²; DA SILVA, V.J.²; OVIDER, I.C.²; LIMA, M.P.O.¹; DOS REIS, T.C.B.²; MATSUMURA, E.L.M.²; EVANGELISTA, G.C.M.²; DE SOUZA, T.A.M.²; DE SALLES, É.M.²; COUTO, S.C.F.³; OLIVEIRA, T.G.M.³; ALMEIDA, R.R.²; ROCHA, V.G.²; RAMOS, R.N.¹. 1. INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. HOSPITAL DAS CLÍNICAS - HCFMUSP, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. FUNDAÇÃO PRÓ-SANGUE HEMOCENTRO DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

In the tumor microenvironment, macrophages are key immune infiltrate components, often playing immunosuppressive and pro-tumoral roles. High densities of CD163+ tumor-associated macrophages (TAMs) correlate with poor prognosis and promote tumor growth, angiogenesis, and metastasis in many tumor types. Although challenging, some studies show macrophages can be genetically manipulated in vitro using CRISPR-Cas9 to modulate target genes. This project aims to reprogram monocyte-derived macrophages via CRISPR-Cas9 to shift them from tumor-promoting to pro-inflammatory profiles. In the first model we used THP-1 cells differentiated with Phorbol 12-myristate 13-acetate (PMA) for 24 hours or kept in culture for 6 days. Flow cytometry analysis revealed a higher viability and up-regulated expression of CD14, CD16, CD86, CD163, and CD206 when PMA was washed-out after 24h. The second model used was the macrophages derived from healthy donors' circulating monocytes. Conditioned media from SKBR3 breast tumor cell cultures' supernatant at 20-30% induced an M2 phenotype with a notable CD163 expression after 7 days of culture. To test transduction feasibility, M2 macrophages (M-CSF + IL-4) were transduced with GFP lentivirus and VPX lentivirus as a transduction enhancer. About 80% of macrophages expressed GFP after 4 days, with no changes in cell viability. Lastly, we tested lentivirus with Cas9 and two guide RNAs targeting CD163 along with VPX lentivirus in monocytes treated with M-CSF for 3 days. By flow cytometry we noted a slight downregulation of CD163 median fluorescence intensity of CD163-targeting RNA compared to the scramble sequence. However, for both scramble and CD163-targeted guides' RNAs, we noted that lentiviral stimulus induced a reduction in CD163 expression when compared to non-transduced monocytes. Future assays are needed to optimize transduction timing, together with genomic assays like T7 endonuclease or sequencing to check the knock-out efficiency.

Keywords: Monocyte-derived Macrophages;CRISPR-Cas9;Tumor Immunology.

2859 - Category: TUMOR IMMUNOLOGY (TU)

Microbiota remodeling in intestinal and mammary tissues during 4T1 breast cancer progression reveals location-specific dysbiosis and immune modulatory potential

SANTOS, C.S.P.D.O.¹; MASSET, M.E.G.²; GARCIA, D.D.A.²; ALVES, I.N.D.S.²; XAVIER, A.M.¹; SANTO, K.P.D.S.¹; GUERREIRO, M.A.D.S.¹; DOS SANTOS, H.F.¹; MONTEIRO, A.C.¹. 1. UNIVERSIDADE FEDERAL FLUMINENSE - UFF, NITERÓI - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Previous studies from our group demonstrated that, in the 4T1 murine model of triple-negative breast cancer (BC), tumor-specific CD4⁺ T cells expressing RANKL accumulate in the bone marrow prior to tumor cell dissemination, where they initiate early osteolytic disease and promote the formation of a pre-metastatic niche. Meanwhile, increasing evidence shows that the microbiota regulates adaptive immunity, bone remodeling, and tumor microenvironment dynamics, emerging as a potential predictive and prognostic factor in BC progression. However, the impact of tumor progression on the structure and diversity of microbial communities across different anatomical compartments remains poorly understood. In this study, we comprehensively investigated the effect of 4T1 BC on the intestinal, mammary, and tumor-associated microbiota. Female BALB/c mice were orthotopically inoculated with 4T1 cells into the fourth mammary fat pad. Fecal, mammary gland, and tumor samples were collected at pre-determined time points: days 5 (early stage), 12 (intermediate stage with lymph node metastasis), and 25 (advanced stage with full metastatic spread), for 16S rRNA (V4–V5) sequencing and bioinformatic analysis of microbial diversity, composition, and dynamics. Early tumor growth led to significant intestinal microbiota shifts, with loss of eubiosis, community destabilization, and reorganization, highlighting day 12 as a potential therapeutic window. Tumor microbiota was marked by low diversity, Staphylococcus dominance, and ecological simplification, suggesting an immune-evasive niche. In the contralateral mammary gland, compositional changes such as exclusive detection of Ligilactobacillus and depletion of Clostridium suggest tumor-driven systemic effects. The identification of context- and site-specific taxa provides a foundation for future studies investigating the contribution of the microbiota to immune modulation and pre-metastatic niche formation in BC bone progression.

Keywords: Breast cancer;metastatic progression;microbiological biomarkers.

2861 - Category: TUMOR IMMUNOLOGY (TU)

Metabolic reprogramming of T cells with SCFAs in a low-glucose environment as a strategy for optimizing CAR-T cells

DE CARVALHO, J.C.S.¹; BRANDÃO, M.¹; TOMA, B.T.¹; MACHADO, C.B.²; ESTEVES, K.S.¹; FURTADO, I.P.¹; AZEVEDO, P.N.³; SORGI, C.A.³; SHIKU, H.⁴; DE SOUZA, L.E.B.¹. 1. HEMOCENTRO DE RIBEIRÃO PRETO - FMRP/USP, RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DO CEÁRA - UFC, FORTALEZA - CE - BRASIL; 3. DEPARTAMENTO DE QUÍMICA - FFCLRP/USP, RIBEIRÃO PRETO - SP - BRASIL; 4. MIE UNIVERSITY, TSU, JAPAN, TSU - JAPÃO.

CAR-T cell therapy represents an advance in cancer treatment, with remarkable success in hematologic malignancies. However, its efficacy in solid tumors remains limited by barriers such as the immunosuppressive tumor microenvironment (TME). One promising strategy to enhance T cell performance in this context is ex vivo metabolic reprogramming. In this study, we investigated the effects of the short-chain fatty acids (SCFAs) sodium acetate and butyrate on the memory phenotype and mitochondrial function of human T cells activated and expanded under glucose restriction. T cells from healthy donors were activated with anti-CD3/CD28 and cultured with IL-7+IL-15 in either normal glucose concentration (11 mM), low glucose (1 mM), or low glucose supplemented with sodium acetate or butyrate (0.5, 5, or 15 mM). Memory markers (CD45RA, CD62L), activation/exhaustion markers (PD-1, LAG-3, TIM-3), and mitochondrial potential ($\Delta\Psi_m$; rhodamine staining) were evaluated on days 2, 6, and 8 post activation by flow cytometry. Glucose restriction reduced proliferation and the frequency of early T cell memory subsets, with 15% stem cell memory T cells (Tscm, CD45RA⁺CD62L⁺) and 17% central memory T cells (Tcm, CD45RA⁺CD62L⁺). Notably, treatment with 15 mM acetate or 0.5 mM butyrate restored the memory profile to levels similar to the normal glucose group, (45% Tcm and 15% Tscm). After 2 days of activation, SCFA-treated cells showed lower $\Delta\Psi_m$, but by day 8, they displayed enhanced $\Delta\Psi_m$. These results suggest that SCFA conditioning under glucose restriction promotes memory T cell generation and mitochondrial fitness. These results suggest that metabolic conditioning with SCFAs in a restricted glycolytic environment favors the formation of memory T cells and improves mitochondrial fitness. This strategy lays the groundwork for integrating metabolic interventions into CAR-T cell manufacturing protocols to improve their antitumor efficacy, particularly in the context of solid tumors.

Keywords: Advanced Cell Therapy;Immunometabolism;Short-Chain Fatty Acids.

2864 - Category: TUMOR IMMUNOLOGY (TU)

Zoledronic acid reduces primary tumor growth, metastatic dissemination and modulates the pro-tumoral adaptive immune response in the 4T1 breast cancer model

XAVIER, A.M.¹; MASSET, M.E.G.²; GARCIA, D.D.A.²; SANTOS, C.S.P.D.O.³; GUERREIRO, M.A.D.S.³; SANTOS, K.P.D.S.³; ALVES, I.N.D.S.²; CANTO, F.³; MONTEIRO, A.C.³. 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL FLUMINENSE - UFF, NITERÓI - RJ - BRASIL.

Bone metastases are a common and debilitating complication of breast cancer, affecting up to 70% of patients with advanced disease and contributing to morbidity through osteolytic lesions, fractures, and skeletal pain. Their development is not merely a passive process but involves active remodeling of the bone microenvironment, largely mediated by increased osteoclast activity and disruption of the RANK/RANKL/OPG axis. Previous studies from our group demonstrated that tumor-specific CD4⁺ T cells expressing RANKL accumulate in the bone marrow prior to breast tumor cell dissemination, initiating osteolytic remodeling and establishing a permissive niche for metastatic colonization in the 4T1 murine model. Here, we investigated whether treatment with the third-generation bisphosphonate zoledronic acid (ZOL) modulates pro-tumoral immune phenotypes and impacts bone tumor progression. BALB/c J mice orthotopically inoculated with 4T1 tumor cells were treated with a single dose ZOL of (100 µg/kg, i.p.) on day 0. Tumor growth and clinical score were monitored. At endpoint, tumor and spleen weight, lymphoid organ cellularity, and metastases were analyzed. ZOL-treated animals showed significantly reduced tumor volume and marked improved clinical scores. Strikingly, most ZOL-treated animals exhibited complete tumor regression. ZOL increased lymphoid organ cellularity and reduced both frequency and number of CD4⁺RANKL⁺ T cells in draining lymph nodes (LNs). Metastatic burden was also reduced on LNs and lungs. Ongoing experiments will assess the impact of ZOL on systemic and local immune phenotypes, bone metastases, as well as on intestinal microbiota composition using 16S rRNA sequencing. These findings support the antitumor and immunomodulatory potential of ZOL and warrant further investigation into its role in regulating pro-metastatic adaptive immunity and the microbiota-immunity-bone metastases axis.

Keywords: Zoledronic acid; Breast cancer; Immune modulation.

2870 - Category: TUMOR IMMUNOLOGY (TU)

Development of an in vitro immunosuppression assay using natural regulatory T cells for functional evaluation of CAR-T cells

REZENDE, R.R.¹; FURTADO, I.P.¹; DE LIMA, S.C.G.¹; BRANDÃO, M.¹; ESTEVES, K.S.¹; ANTUNES, M.D.S.M.¹; SHIKU, H.²; DE SOUZA, L.E.B.¹. 1. HEMOCENTRO DE RIBEIRÃO PRETO - FMRP/USP, RIBEIRÃO PRETO - SP - BRASIL; 2. MIE UNIVERSITY, TSU - JAPÃO.

T cell therapy using lymphocytes engineered to express chimeric antigen receptors (CAR) has shown high remission rates in leukemias and lymphomas. However, its efficacy against solid tumors, responsible for the majority of cancer-related deaths, remains limited. This is mainly due to the dense immunosuppressive tumor microenvironment (TME), enriched with inhibitory molecules and regulatory immune cells, particularly regulatory T cells (Tregs). While Tregs play a critical role in immune homeostasis, their accumulation in the TME impairs antitumor responses and is associated with poor prognosis in cancers such as glioblastoma. Due to the limited availability of in vitro models that accurately recapitulate this immunosuppressive niche, we propose the development of a co-culture assay incorporating natural Tregs to assess their ability to suppress anti-GD2 CAR-T cell activity against glioblastoma cells, a solid tumor that highly express the GD2 antigen. Coculture assays were performed using anti-GD2 CAR-T cells, T98G glioblastoma cells expressing luciferase and natural Tregs isolated from peripheral blood mononuclear cells (PBMCs) by immunomagnetic selection (anti-CD4/CD25). Cocultures were set in duplicates at a 1:1 CAR-T:T98G ratio, and Tregs were added at 0.25 and 0.50 ratios relative to CAR-T cells. Tumor cell death was assessed by quantifying bioluminescence emitted by tumor cells using the IVIS Lumina system at 0h and 72h. After 72 hours, wells containing Tregs showed significantly less tumor cell death, demonstrating a suppressive effect on CAR-T antitumor activity. These results confirm the immunosuppressive effect of natural Tregs on anti-GD2 CAR-T cell activity against glioblastoma in vitro. This co-culture system offers a relevant model to study Treg-mediated inhibition and may support the development of strategies to enhance CAR-T efficacy in solid tumors.

Keywords: Immunosuppression; Regulatory T cells (Tregs); CAR-T cells.

2880 - Category: TUMOR IMMUNOLOGY (TU)

CD39 and CD73 differentially expressed in tumor and free-tumor tissue of colorectal cancer patients - a mechanism of immunosuppression?

ROLIANO, G.G.¹; HOPPEN JUNIOR, A.¹; MARTINS, S.D.F.¹; FRETES, M.¹; AZAMBUJA, D.¹; HERRMANN, F.¹; CARDOZO, A.K.²; BUENO, F.F.²; VISIOLI, F.³; KALIL, A.N.¹; BRAGANHOL, E.¹. 1. UFCSPA, PORTO ALEGRE - RS - BRASIL; 2. SANTA CASA DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL; 3. UFRGS, PORTO ALEGRE - RS - BRASIL.

Introduction: Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related death. Innate immune cells are abundant in the gut and play a key role in immune homeostasis. However, this balance is often affected in the tumor microenvironment, where tumor-associated macrophages (TAM) frequently promote tumor progression. Macrophages, like other immune cells, express purinergic signaling components that mediate communication between cells. This signaling pathway converts ATP, a pro-inflammatory molecule, into adenosine, an immunosuppressive molecule. Although the purinergic system has been implicated in several solid tumors, its role in CRC remains poorly understood. **Methodology:** Formalin-fixed paraffin-embedded (FFPE) tissues from 40 CRC patients treated at Santa Casa de Porto Alegre Hospital (CEP: 3.434.086) were included. Tumor and matched adjacent non-tumor tissues were analyzed from each patient. Histological grading (scores 1–4), tumor-infiltrating lymphocytes (TILs, scores 1–3), and immunohistochemical staining for CD39, CD73, CD68, and CD163 were evaluated by a pathologist. **Results:** Most samples were tumor grade 1 (52.5%), followed by grade 2 (37.5%) and grade 3 (10%). TIL scores were 47.5% (score 1), 35% (score 2), and 17.5% (score 3). CD39 expression was significantly higher in non-tumor tissues, while CD73 expression was significantly elevated in tumor tissues. No significant differences were observed in CD68 or CD163 expression between tumor and non-tumor samples. **Conclusion:** Our findings suggest a change in the purinergic profile within the CRC tumor microenvironment, with increased CD73 expression and reduced CD39 expression in tumor tissues, potentially contributing to immune evasion. The unchanged levels of CD68 and CD163 suggest that macrophage presence and polarization remain stable, indicating that purinergic modulation may occur independently of macrophage phenotype.

Keywords: Colorectal cancer;Purinergic signaling;Macrophages.

2881 - Category: TUMOR IMMUNOLOGY (TU)

CD4⁺ RANKL⁺ T cells are induced in the EO771B (ER⁺PR⁺) breast cancer model that spontaneously metastasizes to bone

GARCIA, D.D.A.¹; MASSET, M.E.G.¹; GUERREIRO, M.A.D.S.²; SANTO, K.P.D.S.²; CANTO, F.²; MONTEIRO, A.C.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL FLUMINENSE (UFF), RIO DE JANEIRO - RJ - BRASIL.

Despite advances in diagnosis and treatment, breast cancer (BC) remains the most frequent and fatal malignancy in women worldwide. Once diagnosed, patients face a ~70% chance to develop bone metastases (BoMet), which are incurable and often associated with severe bone loss, debilitating pain and high risk of pathological fractures, which reduces their quality of life. In the last decade, the knowledge that the metastatic bone colonization is preceded by changes in bone microenvironment emerged, suggesting that some changes in distal sites could establish a pre metastatic niche that support tumor cell engraftment. Using the 4T1 triple-negative BC model, our group previously demonstrated that tumor-specific CD3⁺ RANKL⁺ T cells are critical players in this process, inducing early osteolytic remodeling prior to detectable BoMet. We also showed that the presence of CD19⁺ RANKL⁺ B cells amplify this dysregulation, reinforcing bone loss and metastatic progression. To investigate whether similar immune phenotypes emerge in hormone receptor-positive tumors, we established the EO771B murine model (ER⁺PR⁺HER2⁺), which also spontaneously metastasizes to bone. After orthotopic inoculation of EO771B cells into the mammary fat pad of syngeneic mice, we characterized lymphocyte populations in tumor-draining lymph nodes (tdLN), bone marrow (BM), and tumor-infiltrating lymphocytes (TILs) at 11 and 25 days post-tumor inoculation (dpi). Our analysis revealed a significant increase in RANKL⁺ lymphocytes, particularly within the CD3⁺CD4⁺ T cell compartment in tdLN and BM, when compared to healthy controls. These findings demonstrate that EO771B tumors induce a RANKL⁺ CD4⁺ T cell phenotype, similar to that previously observed in 4T1, suggesting a potential shared immunological signature linked to BoMet across distinct BC subtypes. This phenotype may represent a valuable immunological marker for predicting BoMet and guiding future therapeutic interventions.

Keywords: Bone metastases;Tumor-induced immunophenotypes;CD4⁺ RANKL⁺ T cells.

2896 - Category: TUMOR IMMUNOLOGY (TU)

Development of Anti-CAIX CAR-Monocytes with Secretion of PD-L1 Blocking Antibodies

DE SOUZA, L.L.¹; PIVETTA, R.S.²; SCOTTON, C.V.¹; SUAREZ, E.R.¹. 1. UNIVERSIDADE FEDERAL DO ABC, SANTO ANDRÉ - SP - BRASIL; 2. UNIFESP, SÃO PAULO - SP - BRASIL.

Introduction. Immunosuppression and inefficient migration and trafficking of chimeric antigen receptor (CAR) T cells limit their efficacy for solid tumors (ST). The interaction between inhibitory molecules overexpressed by tumors and their receptors on T cells, such as the programmed cell death ligand 1 (PD-L1) and receptor 1 (PD-1) pair, participates in this immunosuppression and can be blocked by antibodies. Macrophages are a promising alternative cell type for use in CAR therapy (CAR-M) due to their intrinsic ability to infiltrate ST and perform phagocytosis. However, macrophage expansion represents a significant obstacle, and cell lines such as THP-1 are viable option for standardization and *in vitro* studies. Carbonic anhydrase IX (CAIX) is overexpressed in hypoxic ST and constitutively in clear cell renal cell carcinoma (ccRCC). We aim to engineer Anti-CAIX CAR-M secreting anti-PD-L1 antibodies and compare their antitumor and immune checkpoint blockade effects *in vitro* in clear cell renal cell carcinoma (ccRCC). **Methods.** Lentiviral vectors were produced by transient transfection of plasmids into 293T cells using polyethyleneimine. CAR-M cells were characterized by flow cytometry and are being prepared for *in vitro* testing with ccRCC cells. **Results.** Varying cell densities yielded a wide range of CAR expression (40-90%). Using different MOIs, THP-1 viability ranged from 40 to 80% and transgene expression was greater than 65%. Even with short transduction times, CAR expression was detectable in the cells. **Conclusion.** Cell density directly influenced transduction efficiency, while adjustments to the MOI maintained cell viability with high transgene expression. Furthermore, reduced vector exposure times promoted efficient CAR expression, highlighting the potential for protocol optimization with time and input savings.

Approved by: CEP/UFABC CAAE 71055617.3.0000.5594; CIBio/UFABC CQB 0304/10 001/2017; Supported by: FAPESP n°2023/03631-3 and PIBIC/Unifesp.

Keywords: CAR M;cell therapy;immune checkpoint blockade.

2901 - Category: TUMOR IMMUNOLOGY (TU)

OPG-producing B Cells and RANKL-expressing T cells define immune signatures predictive of bone metastases in breast cancer

MONTEIRO, A.C.¹; GARCIA, D.D.A.²; FONTÃO, A.P.A.³; DUROCHER, B.³; MASSET, M.E.G.²; ALVES, I.N.D.S.⁴; CARVALHO, L.G.⁵; DE ANDRADE, C.V.⁶; FIDALGO, G.⁷; COLAÇO, M.V.⁷; NOGUEIRA⁷, L.P.⁸; GAUI, M.D.F.D.⁹. 1. UFF, RIO DE JANEIRO - RJ - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. FIOCRUZ (OSWALDO CRUZ INSTITUTE), RIO DE JANEIRO - RJ - BRASIL; 4. UFF - UNIVERSIDADE FEDERAL FLUMINENSE (LABORATORY OF OSTEO AND TUMOR IMMUNOLOGY, DEPARTMENT OF IMMUNOBIOLOGY, FLUMINENSE FEDERAL UNIVERSITY, RIO DE JANEIRO, BRAZIL) E UFRJ - UNIVERSIDADE FEDERAL DO RJ, RIO DE JANEIRO - RJ - BRASIL; 5. UFF, NITERÓI - RJ - BRASIL; 6. DEPARTMENT OF PATHOLOGY, INSTITUTO FERNANDES FIGUEIRA, OSWALDO CRUZ FOUNDATION, RIO DE JANEIRO - RJ - BRASIL; 7. LABORATORY OF APPLIED PHYSICS TO BIOMEDICAL AND ENVIRONMENTAL SCIENCES, PHYSICS INSTITUTE, STATE UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO - RJ - BRASIL; 8. ORAL RESEARCH LABORATORY, INSTITUTE OF CLINICAL DENTISTRY, U, OSLO - NORUEGA; 9. INTERNAL MEDICINE, FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

We identify a non-classical regulatory role for CD19⁺ B cells in the modulation of bone remodeling and breast cancer (BC) bone metastatic progression. Using models of metastatic 4T1 and non-metastatic BC, we show that 67NR-bearing mice exhibit increased trabecular bone mass associated with expression of osteoprotegerin (OPG) by CD19⁺IgD⁺IgM⁺CD138⁻ B cells. Functional assays demonstrate that 67NR-primed B cells suppress osteoclast differentiation and bone resorption *in vitro* and counteract 4T1-specific T cell-mediated osteolytic activity *in vivo*. Adoptive transfer of 67NR-primed CD19⁺ B cells into 4T1-bearing immunocompetent and immunodeficient hosts inhibits metastatic dissemination and limits primary tumor growth. Notably, these effects are independent of T cell co-transfer but require T cell licensing for acquisition of the OPG-producing phenotype. Genetic silencing of OPG abrogates the regulatory function of 67NR B cells, highlighting its essential role in their effector program. Mechanistically, B cells must be transferred at early stages of tumor progression to retain their therapeutic potential. qPCR and ELISA analyses supported the enrichment of RANKL⁺CD4⁺ T cells in both the tumor microenvironment and lymphoid compartments, whereas OPG⁺CD19⁺ B cells were detected exclusively within the tumor microenvironment. Finally, in a retrospective cohort of human triple-negative BC samples, high RANKL⁺CD3⁺ T cell infiltration correlated with the presence of bone metastases, whereas OPG⁺CD20⁺ B cell infiltration was predominantly observed in patients who remained free of skeletal dissemination. Transcriptomic data from The Cancer Genome Atlas (TCGA) cohort further support the prognostic relevance of these immune phenotypes. Together, our findings uncover a B cell-driven immunological axis capable of restraining osteolytic disease and tumor progression, with implications for early immunological stratification and metastases-preventive interventions in BC.

Keywords: bone metastases;RANKL+CD4+ T cells;OPG+CD19+ B cells.

2934 - Category: TUMOR IMMUNOLOGY (TU)

Effectiveness and molecular mechanisms of extracellular vesicles in therapy of acute myeloid leukemia: systematic review

DE OLIVEIRA, L.D.; DE ALMEIDA, J.A.; DOMINGUES, V.T.; IAMAMOTO, K.T.; RODRIGUES, A.F.F.; MORI, G.G..
UNIVERSIDADE DO OESTE PAULISTA, PRESIDENTE PRUDENTE - SP - BRASIL.

Acute myeloid leukemia (AML) is the most common leukemia in adults and has a low survival rate, progressing rapidly and becoming fatal if not treated. Although multiple therapies are available, their effectiveness remains limited. Extracellular vesicles (EVs), which serve as immunomodulatory molecules, have emerged as a promising alternative for treating several diseases, including AML. The objective of this study was to evaluate the effectiveness of EV therapy in treating AML. A systematic review was conducted by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, utilizing searches in PubMed/MEDLINE, Scopus, Embase, and the Cochrane Library databases up to September 2024. In vivo studies that answered the PICO question "Is EV therapy effective in the treatment of AML?" were included. The outcomes considered were the therapeutic effectiveness and the molecular mechanisms involved. Risk of bias was assessed using the SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) tool. Out of 910 articles analyzed, 10 were included. Seven studies demonstrated effectiveness; one reported results comparable to cell therapy. Effective EVs were derived from mesenchymal stem cells, NK cells, or modified leukemic cells. EVs from leukemic cells carrying miR-92a and miR-548ac had unfavorable effects. The molecular mechanisms involved the modulation of STAT3, RAF1, and miR-34c-5p, along with the inhibition of FLT3-ITD and miR-125b. Five studies provided high-quality evidence. Further randomized clinical trials in humans are necessary to confirm these findings. In conclusion, EVs hold significant therapeutic potential for AML, especially when combined with specific molecular strategies and selected cell sources.

Keywords: Extracellular Vesicles;Exosome;Acute Myeloid Leukemia.

2938 - Category: TUMOR IMMUNOLOGY (TU)

Impact of intestinal infection on development of cancer in barrier tissues

COELHO, M.P.S.; GONÇALVES, L.M.; DA CONCEIÇÃO, L.B.; CAMARGO, L.; DE ARAUJO, M.V.; ALVES, C.B.; DE ABREU, A.M.; DA SILVA, G.W.; OLIVEIRA, B.D.C.; RODRIGUES, G.M.B.; CARDOSO, A.; DA FONSECA, D.M.; DE ASSIS, J.B.. ICB-USP/INCT, SÃO PAULO - SP - BRASIL.

Barrier tissues are sites of intense immunological communication, mediated by the trafficking of immune cells and microbial products. Consequently, disturbances in one barrier tissue can have repercussions in others. Pathogens are common sources of such disturbances, often with long-lasting effects. In the Laboratory of Mucosal Immunology, we investigate how acute gastrointestinal infection impacts gut mucosal homeostasis, using *Yersinia pseudotuberculosis* (YP) as a model. Our findings show that, even after infection clearance, the gut immune tone is altered, resulting in chronic inflammation and impaired capacity of regulatory T cells (Tregs) induction, which are critical for maintaining immune balance in the gut. This Treg dysfunction appears to confer protection against colorectal cancer in previously infected mice. However, these mice unexpectedly develop lung tumors, suggesting cross-talk between intestinal and pulmonary tissues. Based on these observations, the aim of this work is to evaluate whether a previous YP infection increases susceptibility to tumor development in other barrier tissues, such as the lung. C57BL/6 mice previously infected with YP and later instilled intratracheally with Lewis Lung Cancer cells showed nuclear irregularities, architectural disruption, extensive inflammatory infiltrate, and angiogenesis in the lungs. In contrast, uninfected animals exhibited some inflammation but preserved lung structure, suggesting that previously infected mice could be more prone to lung cancer. In parallel, by using a model of melanoma induced by the injection of B16-F10 cells, we found that previously infected mice seem to develop larger tumors and present a higher tumor-to-body weight ratio compared to uninfected controls. Although initial, our findings highlight the communication between barrier tissues and suggest that prior YP infection may increase susceptibility to tumor development in other barrier tissues, such as the lung and skin.

Keywords: cancer;Treg;infection.

2945 - Category: TUMOR IMMUNOLOGY (TU)

THE IMMUNOMODULATORY EFFECTS OF THE CHRONIC EXPOSURE TO BUTYRATE ON CERVICAL CANCER MICROENVIRONMENT

POMBO, F.C.D.N.¹; COLUNNA, F.C.¹; LEPIQUE, A.P.². 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. COMPREHENSIVE CENTER FOR PRECISION ONCOLOGY (C2PO) - USP, SÃO PAULO - SP - BRASIL.

Cervical cancer (CC) is a globally prevalent tumor caused by Human Papillomavirus (HPV) infection, though other risk factors are important for cancer development, such as changes in the vaginal microbiota. The decrease in lactate-producing *Lactobacillus* and the increase in short-chain fatty acids (SCFAs) producing anaerobic bacteria may be part of the mechanisms leading to carcinogenesis. Butyrate, a SCFA, activates the GPR41, GPCR43, and GPR109A receptors, and inhibits histone deacetylase (HDAC), thus modulating cell metabolism and proliferation in immune and tumor cells. In CC, data shows that butyrate has an ambiguous role: protumoral, through immunosuppression, and antitumoral, through tumor cell apoptosis. Although generally considered a regulator of immune responses, there is evidence that butyrate can trigger microbicide activity in macrophages. We hypothesized that butyrate could modulate macrophages in the tumor microenvironment and play a role in HPV mediated carcinogenesis. Therefore, our objective was to test butyrate treatment effects on cells immortalized by HPV oncogenes in coculture with leukocytes. We used immortalized human keratinocytes transduced with E6 and E7 (HaCaT E6E7) or a control cell line (HaCaT pLXSN) susceptible or butyrate resistant (HaCaT E6E7B, HaCaT pLXSNB) and a cancer derived cell line, SiHa, in individual cultures or co-cultures with peripheral blood mononuclear cells (PBMCs). We observed that, although butyrate promoted cell death, butyrate-resistant cells showed higher proliferation rates than the original cell lines. In co-cultures, preliminary results suggest that macrophages in coculture with SiHa treated with butyrate have a decreased HLA-DR and CD80 expression, pointing to a less activated macrophage phenotype. In conclusion, our results indicated that butyrate may promote the development of more resilient tumors, however, through finer approaches, we desire developing a more robust understanding of butyrate's role in CC.

Keywords: Immunomodulation;Cervical Cancer;Butyrate.

2947 - Category: TUMOR IMMUNOLOGY (TU)

Optimization of NSG and NBSGW Mouse Models Humanized with Human CD34⁺ Hematopoietic Stem Cells for Preclinical Cancer Immunotherapy Studies

SALLES, P.P.Z.¹; CAVALCANTE, M.D.S.¹; FEITOZA, J.M.S.¹; DE LIMA, F.M.D.S.¹; DE BARROS, L.A.K.¹; GUIMARÃES, F.²; ONO, L.A.R.²; DO NASCIMENTO, M.L.C.²; SEABRA, M.V.C.². 1. BOLDRINI RESEARCH CENTER (BRC); UNIVERSITY OF CAMPINAS (UNICAMP), CAMPINAS - SP - BRASIL; 2. PROF. DR. JOSÉ ARISTODEMO PINOTTI WOMEN'S HOSPITAL (CAISM), CAMPINAS - SP - BRASIL.

Humanized mouse models generated by transplantation of human CD34⁺ hematopoietic stem cells (HSCs) are powerful tools for studying the human immune system and evaluating novel immunotherapies. This study aimed to establish and optimize humanization protocols in NSG and NBSGW mice for downstream application in preclinical models of pediatric leukemia and therapeutic antibody testing, with a focus on translational cancer research. HSCs were derived from umbilical cord blood (UCB) collected at CAISM/UNICAMP and commercial sources (bone marrow, mobilized peripheral blood, and UCB). Mice were transplanted intravenously or intrahepatically. NSG mice received sublethal irradiation (2.0 or 2.5 Gy), while NBSGW mice required no conditioning. The 2.0 Gy dose provided a balance between effective bone marrow ablation and reduced mortality. Humanization ($\geq 25\%$ hCD45⁺ in peripheral blood) varied across conditions: 100% in adult NSG mice (n=4) using CAISM-derived UCB, 25% in adult NBSGW (n=8), 85.7% in neonates (n=7), and positive in a single juvenile (n=1). For commercial HSCs, NBSGW neonates achieved 100% humanization with UCB (n=7) and bone marrow (n=8), while NSG neonates (n=8) did not reach the threshold. B cells predominated in all groups, and optimization of cell purity remains critical, as low-purity CAISM-derived HSCs (15.7–62.5% hCD34⁺) may contribute to GvHD risk. Volume constraints in hospital-collected UCB also represent a technical barrier. Notably, neonatal NBSGW mice demonstrated superior humanization efficiency, suggesting a preferred model for future studies. Ongoing work includes enhancing HSC collection and purification and implementing these models in therapeutic evaluation of humanized anti-IL7R α antibodies for pediatric leukemia, thus reinforcing their relevance in tumor immunology. CEUA: 0045/2024; 0051/2024. CEP: CAAE 59137022.9.0000.5376.

Keywords: humanized mice;CD34⁺ HSCs;preclinical model.

2951 - Category: TUMOR IMMUNOLOGY (TU)

Enhancement of immune checkpoint therapy through targeting myeloid cells

CEDRÉS, C.; DUARTE, D.; MORENO, M.; GONZALEZ, G.. UNIVERSIDAD DE LA REPÚBLICA (URUGUAY), MONTEVIDEO - URUGUAI.

Recent investigations in murine tumor models have demonstrated that PD-L1 blockade on antigen-presenting cells (APCs) drives the antitumor response. This promotes the sustained activation of progenitor-exhausted CD8⁺ T cells, thereby supplying the tumor with effector cells that, although inheriting an exhausted phenotype, retain potent antitumor activity. At the same time, it has been proven that immune suppression in the tumor microenvironment (TME), caused by different populations of myeloid cells, directly impacts the response to these therapies. Building on these findings, we explored the enhancement of anti-PD-L1 antibodies by directing them to APCs or myeloid cells as a whole, combining them with antibodies targeting different immune receptors. To this end, we generated bispecific antibodies based on nanobodies. The antitumor effect was evaluated in murine models of colon carcinoma. We documented slower tumor growth and extended survival in mice treated with our constructs under different treatment regimens compared to the control groups. This tumor growth delay was associated with changes in the immune profile of the TME and tumor-draining lymph nodes (TDLNs), as revealed by immunophenotyping. Furthermore, we observed a sustained antitumor effect in a PD-L1 knockout tumor model, indicating that the therapeutic activity is mediated primarily through PD-L1 blockade on immune cells rather than tumor cells. Hence, we conclude that the simultaneous binding of bispecific antibodies to PD-L1 and myeloid cells allows for a focused blockade of the PD-1/PD-L1 axis on these critical regulatory cells, thereby enhancing the effectiveness of checkpoint immunotherapy.

Keywords: Immune checkpoint blockade;PD-L1;Nanobodies.

2959 - Category: TUMOR IMMUNOLOGY (TU)

Toward metabolically fit CAR-T cells: inosine supplementation increases antitumor efficacy against glioblastoma cellsESTEVEZ, K.S.¹; FURTADO, I.P.¹; BRANDÃO, M.¹; DE LIMA, S.C.G.¹; DE QUADROS, A.U.¹; MACHADO, C.B.²; DE CARVALHO, J.C.S.¹; SHIKU, H.³; DE SOUZA, L.E.B.¹. 1. FUNDAÇÃO HEMOCENTRO DE RIBEIRÃO PRETO, FACULDADE DE MEDICINA DE RIBEIRÃO PRETO/USP, RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DO CEARÁ (UFC), FORTALEZA - CE - BRASIL; 3. MIE UNIVERSITY, TSU - JAPÃO.

Despite its success in certain hematologic malignancies, chimeric antigen receptor (CAR)-T cell therapy still faces significant challenges in treating solid tumors. One promising approach to improve efficacy is the generation of CAR-T products enriched in early memory T cell subsets, which are strongly associated with favorable clinical outcomes, and can be achieved by modifying culture conditions. In this study, we aimed to optimize the expansion of CAR-T cells to produce a less differentiated cellular product with enhanced cytotoxic potential for the treatment of glioblastoma and other GD2-expressing tumors by incorporating inosine, an immunomodulatory nucleoside, in the culture medium. Anti-GD2 CAR-T cells were generated from T lymphocytes, obtained from two healthy donors, and expanded for 8–9 days under three conditions, all stimulated by IL-7 and IL-15: (1) inosine-containing medium (INO group); (2) glucose-containing medium for 4 days, followed by inosine-containing medium (GLU/INO group); and (3) glucose-containing medium throughout (GLU group). Our manufacturing process resulted in consistent CAR expression levels (23.85±3.30%), assessed 5-6 days post-transduction. All CAR-T cells exhibited potent cytotoxic activity against GD2⁺ T98G glioblastoma cells after 48h of coculture at a 1:8 effector-to-target ratio. Notably, cells cultured with inosine (INO and GLU/INO groups) showed higher tumor-killing activity (82.07±1.78% and 84.72±4.16% lysis, respectively) compared to those expanded in glucose alone (GLU group: 70.36±8.77% lysis; p<0.05), accompanied by the secretion of higher levels of granzyme B and TNF-alpha compared to cells cultured solely in glucose. Future work will focus on further characterizing memory T cell subsets, in vivo functional assessments, and characterizing the metabolic properties of CAR-T cells to support the development of a metabolically optimized cell therapy product with improved persistence and activity against GD2⁺ malignancies.

Keywords: CAR-T cells;Immunotherapy;Inosine.

2960 - Category: TUMOR IMMUNOLOGY (TU)

In vitro investigation of dietary influence on lipid droplets and colon cancer development

RIBEIRO, L.R.¹; SANTOS, N.D.S.¹; LABOURBE, E.²; VIEIRA, B.L.M.D.O.³; BRANCO, J.M.C.R.¹; VIOLA, J.P.D.B.¹. 1. PROGRAM OF IMMUNOLOGY AND TUMOR BIOLOGY, BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. DÉPARTEMENT GÉNIE BIOCHIMIQUE, INSTITUT NATIONAL DES SCIENCES, TOULOUSE, FRANCE, TOULOUSE - FRANÇA; 3. PROGRAM OF IMMUNOLOGY AND TUMOR BIOLOGY, BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

Gastrointestinal (GI) cancer ranks as the third most prevalent cancer type globally and is significantly influenced by inflammation and dietary patterns. The adoption of modern lifestyles and advances in food processing have led to excessive consumption of high-fat and high-sugar foods, contributing to increased caloric intake and lipid accumulation in cells. These changes disrupt normal metabolic pathways and are closely associated with inflammation and unfavorable tumor prognoses. Notably, alterations in lipid metabolism during carcinogenesis impact key metabolic regulators and may represent promising targets for anti-cancer strategies. This study aimed to evaluate the role of lipid droplets (LDs) in modulating tumor aggressiveness and progression *in vitro*. Non-tumor intestinal cells (IEC-6) and three human colorectal adenocarcinoma cell lines (LoVo, HT29, and CACO-2) were treated with oleate and palmitate for 24, 48, and 72 hours. Following treatment, assays for cell viability and proliferation, immunofluorescence staining for LD and proliferation markers, ATP quantification, and ROS production were conducted. Among the tumor lines, CACO-2 cells exhibited the highest proliferative activity at 72 hours and presented a marked increase in both number and size of LDs at 24 hours, with a subsequent decline at later time points. In contrast, IEC-6 cells showed limited capacity to use LDs as an energy source. Similarly, LoVo and HT29 cells accumulated LDs but did not metabolize them efficiently. CACO-2 cells, however, demonstrated effective lipid utilization, suggesting a greater metabolic flexibility and an ability to exploit LDs for energy production. These findings highlight potential metabolic distinctions among tumor cell lines, emphasizing the relevance of lipid metabolism in cancer progression. Further studies are warranted to confirm these observations and to elucidate the precise role of LDs in tumor cell metabolism, inflammation and aggressiveness.

Keywords: Lipid Droplets;Diet-induced carcinogenesis;Tumor progression.

2961 - Category: TUMOR IMMUNOLOGY (TU)

ANALYSIS OF IMMUNOMODULATORY MECHANISMS INVOLVED IN TUMOR PROGRESSION OF HEAD AND NECK SQUAMOUS CELL CARCINOMAS

DE MELLO, I.M.A.; LEPIQUE, A.P.. UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL.

Head and Neck Squamous Cell Carcinomas (HNSCCs) are the sixth most common cancers globally, with rising incidence in younger populations despite being more prevalent in adults over 50. While precursor lesions increase the risk of progression to invasive cancer, most cases are diagnosed at advanced stages. Identifying early diagnostic biomarkers remains a crucial yet challenging task due to the disease's molecular complexity. HNSCCs are etiologically classified into HPV-related (Human Papillomavirus) and tobacco/alcohol-related subtypes, a distinction that influences carcinogenic mechanisms, tumor microenvironment, treatment strategies, and prognosis. Based on this, our main goal is to identify immunological biomarkers and immunomodulatory mechanisms in precursor lesions that could indicate lesions at higher risk of progression to cancer. Initially, we will establish *in vitro* study models based on an immortalized keratinocyte cell line, HaCaT, transduced with HPV16 oncogenes (HaCaT E6/E7) and chronically exposed to cigarette smoke (HaCaT CS). Together with a control cell line, these cells will be used for organotypic cultures to which we will add mixed or isolated leukocytes to mimic precursor lesions and allow us to study the cross-talk between leukocytes and the different microenvironments. We will investigate epithelial cell morphology, proliferation, cytokine profile, and gene/protein expression. In the future, our results will be validated in a cohort of patients with precursor lesions and cancer. We expect to define early signatures of malignant progression, thus contributing to early diagnostic strategies and treatment. This study is funded by the National Council for Scientific and Technological Development (CNPQ 310154/2021-9) and by the São Paulo Research Foundation (FAPESP 2022/10338-5).

Keywords: Tumor microenvironment;Carcinogenesis;Immune Biomarkers

2964 - Category: TUMOR IMMUNOLOGY (TU)

Intrinsic cytotoxic activity of $\gamma\delta$ T cells against hematologic and solid tumors supports their use in allogeneic cell therapy

ANTUNES, M.D.S.M.; BATISTA, M.H.Z.; DE SOUZA, L.E.B.. HEMOCENTRO DE RIBEIRÃO PRETO - FMRP/USP, RIBEIRÃO PRETO - SP - BRASIL.

$\gamma\delta$ T cells are a rare subset of unconventional T lymphocytes that bridge innate and adaptive immunity. They recognize stressed or transformed cells through MHC-independent mechanisms, resembling NK cell activity, and their infiltration in tumors is associated with favorable prognosis. Their ability to exert antitumor effects without causing graft-versus-host disease makes them promising candidates for allogeneic cell therapies. However, the intrinsic cytotoxic potential and biological behavior of $\gamma\delta$ T cells remain insufficiently characterized, limiting their clinical translation. In this study, we evaluated the intrinsic cytotoxic activity of $\gamma\delta$ T cells expanded from peripheral blood mononuclear cells (PBMCs) of three healthy donors. After CD4/CD8 depletion, cells were stimulated with 5 μ M zoledronic acid for 48 hours and cultured for 12 days. $\gamma\delta$ T cell frequency increased from 13.35% $\pm$ 12.02 to 84.14% $\pm$ 9.43, with an average expansion of 37.20-fold ($\pm$ 30.91). Cytotoxicity was assessed *in vitro* through bioluminescence quantification of luciferase-expressing cell lines derived from hematologic (Raji, K562) and solid (T98G, HCT116) tumors at different effector:target (E:T) ratios and timepoints. All targets showed dose- and time-dependent lysis. Raji cells exhibited ~20% lysis at E:T 3:1 after 24h, with sustained lysis only at 9:1 after 48h. K562 cells showed >40% lysis at 6:1 after 24h and from 3:1 after 48h. T98G cells were highly sensitive, with ~40% lysis at 1:1 after 24h and >60% at 1:1 after 48h. HCT116 cells showed >40% lysis only after 48h, starting at 3:1. These results confirm robust, intrinsic cytotoxicity of $\gamma\delta$ T cells and support their use as off-the-shelf effectors in cancer immunotherapy. Further studies are warranted to optimize their clinical application.

Keywords: $\gamma\delta$ T cell; Intrinsic cytotoxicity; Immunotherapy.

2969 - Category: TUMOR IMMUNOLOGY (TU)

Efficient transduction of non-activated T cells using baboon envelope lentiviral vectors and IL-7/IL-15 preconditioning for CAR-T applications

BRANDÃO, M.; FURTADO, I.P.; ESTEVES, K.S.; MACHADO, C.B.; DE CARVALHO, J.C.S.; DE LIMA, S.C.G.; DE SOUZA, L.E.B.. HEMOCENTRO DE RIBEIRÃO PRETO - FMRP/USP, RIBEIRÃO PRETO - SP - BRASIL.

Chimeric antigen receptor (CAR)-T cell therapy has shown remarkable efficacy in B-cell malignancies. However, treatment durability is often hampered by T-cell exhaustion and limited persistence. Naïve T cells offer better expansion and persistence, but conventional VSV-G-pseudotyped lentivirus transduction requires activation, promoting differentiation and reducing cytotoxicity. Cytokines IL-7 and IL-15 allow T cells to exit quiescence without activation. Also, baboon retroviral envelopes (BaEVTR and BaEVRLess) were reported to transduce quiescent cells of different origins. Thus, this study aimed to optimize non-activated T-cell transduction, by assessing the effect of IL-7/IL-15 preconditioning duration and comparing the efficiency of gene transfer using lentiviral vectors (LVs) pseudotyped with the envelopes VSV-G, BaEVTR, and BaEVRLess. Human peripheral T cells were isolated by negative selection and cultured with IL-7/IL-15 (10 ng/mL) for 1-5 days, followed by transduction with EGFP LVs at a different MOIs (1, 5, 10). Cells were assessed for percentage of EGFP+ cells and percentage and absolute numbers of transduced naïve/memory T cell subsets by flow cytometry. Preconditioning for 4–5 days showed higher EGFP+ percentages. BaEVTR and BaEVRLess greatly outperformed VSV-G, especially at MOI 10: BaEVRLess (41.8% $\pm$ 1.4%), BaEVTR (45.4% $\pm$ 1.4%), VSV-G (21.4% $\pm$ 0.7%). Additionally, BaEVTR-LV (35.6%) and BaEVRLess-LV (27.3%) led to higher frequencies of EGFP+ T naïve/T stem cell memory subsets than VSV-G (22.2%). By combining IL-7/IL-15 preconditioning with BaEV-pseudotyped lentiviral vectors, this approach enables efficient genetic modification of non-activated T cells without compromising T-cell stemness. This platform may serve as a foundation for next-generation CAR-T manufacturing protocols aimed at improving persistence and long-term antitumor efficacy.

Keywords: CAR-T cells; Cell therapy; Gene transfer.

2994 - Category: TUMOR IMMUNOLOGY (TU)

Immunogenomic Profiling of Membrane Proteins Identifies CAR-Relevant Targets in Solid Tumors

ALMEIDA, J.M.; DE SOUZA, E.R.; NOGUEIRA, H.A.C.; LOPES, V.D.S.; GARCIA, A.B.; MEDINA-ACOSTA, E.. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE, CAMPOS DOS GOYTACAZES - RJ - BRASIL.

Immunotherapy has become a promising strategy by enhancing the immune system's ability to recognize and eliminate tumor cells. Among these, chimeric antigen receptor (CAR)-engineered cells—such as CAR-T and CAR-NK—represent major advances, though their efficacy in solid tumors remains limited, largely due to the scarcity of specific, accessible surface targets. Plasma membrane proteins at the tumor-immune interface have thus emerged as key candidates to expand the immunotherapeutic target repertoire. Here, we performed a systematic multi-omic analysis of 9,937 membrane protein-coding genes across 33 cancer types to identify clinically relevant biomarkers. Six omic layers (mRNA, transcript, and protein expression; somatic mutations; copy number alterations; and CpG methylation) were integrated and correlated with immune-relevant tumor phenotypes, including microsatellite instability (MSI), tumor mutational burden (TMB), and stemness. Significantly associated signatures (adjusted $p < 5e-8$) were assessed for prognostic impact across four survival endpoints (OS, DSS, DFI, PFI) and linked to tumor-infiltrating immune profiles, classifying the tumor microenvironment (TME) as hot, cold, or variable, with anti-, pro-, or dual immune phenotypes. We identified 43,860 multi-omic signatures; one, comprising transcripts ENST00000309285 (*XCR1*), ENST00000353334 (*CD74*), and ENST00000547437 (*CLEC17A*), was notably overexpressed in breast cancer, negatively correlated with stemness, and protective across all survival metrics. This signature was associated with a hot TME enriched with anti-tumoral infiltrates, indicating an immunologically active phenotype that contributes to tumor control. *CD74*, *XCR1*, and *CLEC17A* emerged as high-value immunological targets—respectively implicated in antigen presentation, cross-priming, and immune recognition—highlighting their translational potential for designing next-generation CAR-based therapies in solid tumors.

Keywords: Plasma Membrane Proteins;Immunotherapeutic Targets;Multi-omic Signatures.

2996 - Category: TUMOR IMMUNOLOGY (TU)

Autophagy Signatures Define Multi-Omic Immune Landscapes with Therapeutic Implications

LOPES, V.D.S.; DE SOUZA, E.R.; NOGUEIRA, H.A.C.; ALMEIDA, J.M.; MEDINA-ACOSTA, E.. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE – UENF, CAMPOS DOS GOYTACAZES - RJ - BRASIL.

Cancer remains a major global health challenge, requiring therapeutic strategies that address both tumor-intrinsic features and the surrounding immune context. Autophagy, a key catabolic process for cellular homeostasis, plays a dual role in modulating the tumor microenvironment, enhancing or suppressing antitumor immunity depending on disease stage and local conditions. We aimed to define autophagy-related multi-omic gene signatures and assess their association with the tumor immune landscape — including immune cell infiltration and checkpoint expression — to uncover prognostic biomarkers and immunotherapeutic targets across 33 cancer types. We analyzed 2,739 autophagy-associated genes across seven omic layers (mutation, CNV, methylation, mRNA, transcript isoforms, miRNA, and protein), alongside three phenotypic metrics (TMB, MSI, and stemness), which capture neoantigen burden, T cell recruitment, and stem cell-driven immune evasion. Expression and survival analyses were conducted, and signatures were classified according to immune microenvironment phenotype (antitumoral, protumoral, or dual) and immune profile (hot, cold, or variable), based on immune cell abundance estimated via CIBERSORT and xCell. We identified 66 signatures across 11 tumor types: 25.7% were antitumoral/hot; 24.3% antitumoral/cold; 37.9% protumoral/hot; and 12.1% protumoral/cold. All signatures showed significant association with at least one clinical survival metric. In lung adenocarcinoma, the KPNA2 + RAD51 signature acted as a risk factor, despite its association with an antitumoral/hot immune context. Functional data suggest KPNA2 may facilitate immune evasion via PD-L1 upregulation, while RAD51 supports genomic stability and T cell survival. These findings highlight autophagy-related multi-omic signatures as key modulators of the tumor immune microenvironment and potential biomarkers and targets for combinatorial therapies involving autophagy modulation and personalized immunotherapy.

Keywords: Autophagy Immune Signatures;Multi-Omic Biomarkers;Tumor Immune Microenvironment.

3013 - Category: TUMOR IMMUNOLOGY (TU)

Local inhibition of NLRP3 inflammasome promotes OSCC tumor growth and affects immune cell infiltration

PELEGRIIN, Á.F.; NASCIMENTO, C.R.; FERREIRA, T.D.S.; DE CARVALHO, M.M.; PASCHOAL, D.; ALEMISEREJ, F.; REIS, I.B.; ROSSA JR, C.. SÃO PAULO STATE UNIVERSITY, SCHOOL OF DENTISTRY, ARARAQUARA - SP - BRASIL.

Oral squamous cell carcinoma (OSCC) is an aggressive malignancy with high mortality and recurrence rates. The tumor microenvironment (TME) including various inflammatory cells and mediators has been targeted therapeutically as it influences disease progression. The NLRP3 inflammasome has emerged as an important regulator of immune response in cancer, although its effects in OSCC are unclear. This study investigates the role of NLRP3 in OSCC progression using an orthotopic (floor-of-mouth) syngeneic murine model. Experimental tumors were induced by injection of MOC2 cells in C57BL/6 wild-type (WT) and NLRP3 knockout (KO) mice. Additionally, local inhibition of NLRP3 was performed by intra-tumoral injections of MCC950 (50 uL of a 1 mM solution) in WT and NLRP3 KO mice. After 14 days, tumors were collected for morphological and flow cytometry analyses. The absence or inhibition of NLRP3 resulted in larger tumors. Flow cytometry of dissociated tumors revealed increased overall prevalence of CD45+ cells after MCC950 treatment in both WT and KO animals, with a distinct increase of CD4+ T lymphocytes. NLRP3 KO mice had fewer M2-like macrophages (CD11b+F4/80+CD206+) compared to WT mice, regardless of MCC950 treatment. In tumor cells (CD45-), MCC950 treatment increased expression of CD47, a "don't eat me" signal involved in immune evasion. In conclusion, NLRP3 has pro-tumoral roles in OSCC, as disruption of its function generated larger and more leukocyte-infiltrated tumors with enhanced immune evasion.

Keywords: CANCER;INFLAMMASOMES;NLRP3.

3014 - Category: TUMOR IMMUNOLOGY (TU)

Tumor microenvironment of colon carcinoma: cross-modulation between stromal and immunological components in a metabolically altered system

MASCARENHAS, G.D.; GALDINO, M.E.F.; DE LIMA, B.C.; DA CUNHA, F.N.; DE ARAUJO, W.G.; DE MELO, C.B.; WERNECK, M.B.D.F.; DIAZ, B.L.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Colorectal carcinoma (CRC) is one of the most prevalent and lethal tumors worldwide, with a unique tumor microenvironment (TME) with characteristic stromal content, energy metabolism, and immune cell infiltration. Among the immunological components present in CRC's TME, mast cells (MCs) are resident cells of connective tissues and mucosal surfaces, with a well-defined role in allergic contexts but still unclear functions in tumor biology. MCs can be found within the tumor stroma or in hypoxic and metabolically altered regions where increased intratumoral lactate and acidification of the TME are also found. However, the effects of lactate on MC responses have been scarcely studied. Our objective is to understand how the interaction between metabolic, stromal, and immune components can affect tumor progression. Organoids were established by plating 1000 murine colon carcinoma cells (MC38 cell line) in the absence or presence of 9000 fibroblasts (NIH/3T3 cell line). To induce metabolic stress, 40 mM lactate was added at the time of organoid establishment. After two days, 1000 bone marrow-derived mast cells (BMMCs) were added. Initially, we observed that fibroblasts negatively affect organoid growth in a cell-cell interaction-dependent manner. We also observed that our organoids exhibit reduced growth rates when cultured with lactate, and MCs appear to positively affect this growth. Additionally, co-culture with MCs without lactate addition seems to increase the migratory capacity only in organoids cultured with fibroblasts. However, when lactate is added, this increase is also observed in organoids without fibroblasts. Together, these data suggest that the addition of MCs confers a protective role for the organoid, which may vary depending on the composition of the microenvironment. We intend to understand how MCs may be involved in the growth and migratory capacity of these cells, as well as how lactate addition may modify the expression of these molecules.

Keywords: Tumor microenvironment;Mast cell;Lactate.

3021 - Category: TUMOR IMMUNOLOGY (TU)

Multi-Optosis Mapping of CAR-Target Signatures Uncovers Immunogenomic Drivers of Tumor Immune Phenotypes

DE SOUZA, E.R.; NOGUEIRA, H.A.C.; LOPES, V.D.S.; MEDINA-ACOSTA, E.. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE, CAMPOS DOS GOYTACAZES - RJ - BRASIL.

Tumor immune responsiveness is shaped by a complex interplay between intrinsic genomic alterations, extrinsic immune cues, and regulated cell death (RCD) pathways that collectively remodel the tumor microenvironment (TME). Chimeric antigen receptor (CAR) T-cell therapies represent a frontier in cancer treatment, yet most CAR targets have been studied without considering their broader immunogenomic context or associations with RCD. Mapping these relationships may uncover novel, clinically relevant candidates for immunotherapy development. To characterize this landscape, we developed a multi-optosis model integrating 25 distinct forms of RCDs with seven omic layers—mutations, CNVs, CpG methylation, mRNA expression, transcript isoforms, miRNA, and protein—across 33 cancer types. These data were correlated with immunogenomic phenotypes defined by Microsatellite Instability (MSI), Tumor Mutation Burden (TMB), Tumor Stemness, clinical survival outcomes, and computationally deconvoluted profiles of immune infiltration context. We identified 879 multi-omic signatures containing at least one component classified as a CAR target. Of these, five were associated with unfavorable clinical outcomes and immunosuppressive features, whereas four displayed favorable prognostic profiles and immune activation. The signature defined by mutations in *FAP* and *PXN* in HNSC showed a positive correlation with TMB and MSI, representing a risk factor for OS and DSS survival metrics, and was associated with a pro-tumoral and immunologically “cold” TME. Conversely, the signature composed of mutations in *BAG4*, *CD2*, and *TNFRSF9* in COAD showed a positive association with TMB and exhibited a protective effect on OS, DSS, and PFI, corresponding to an antitumoral and immunologically “hot” phenotype. This study presents an immunogenomic and multi-omic landscape of CAR target-associated signatures shaped by regulated cell death pathways in cancers, revealing promising candidates for immunotherapy.

Keywords: CAR-Target;Immunogenomics;Regulated Cell Death.

3039 - Category: TUMOR IMMUNOLOGY (TU)

Regulation of Carbonic Anhydrase IX and Cyclooxygenase-2 in Renal Cell Carcinoma and Its Impact on the Efficacy of CAR T CellsDE CAMPOS, N.S.P.¹; PIVETTA, R.S.¹; DE SOUZA, L.L.²; KINKER, G.S.³; MEDINA, T.³; RODRIGUES, T.²; SUAREZ, E.R.². 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SANTO ANDRÉ - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DO ABC, SANTO ANDRÉ - SP - BRASIL; 3. A.C. CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

Clear cell renal cell carcinoma (ccRCC) is the most prevalent form of kidney cancer, with a 5-year survival rate of less than 10% in metastatic cases. Current therapeutic strategies for advanced ccRCC offer only modest improvements in progression-free survival. Chimeric antigen receptor T cells (CAR T cells) targeting carbonic anhydrase IX (CAIX) have reemerged as a promising alternative to ccRCC treatment based on recent preclinical data. Both CAIX and cyclooxygenase-2 (COX-2) are implicated in tumor progression and are overexpressed in ~95% and ~50% of ccRCC cases, respectively. We analyzed CAIX–COX-2 crosstalk in ccRCC and assessed the impact of their modulation on anti-CAIX CAR T cell efficacy. Correlations between CAIX and COX-2 expression were analyzed in silico using RNA-seq data from ccRCC cell lines. The effects of celecoxib (a COX-2 inhibitor), anti-CAIX monoclonal antibodies (mAbs), and anti-CAIX CAR T cells were assessed in vitro by immunofluorescence microscopy and flow cytometry. We identified a positive correlation between CAIX and COX-2 expression in ccRCC cell lines both in silico and in vitro. Notably, COX-2 blockade with celecoxib led to up to a 90% reduction in CAIX expression, while treatment with two different anti-CAIX monoclonal antibodies (mAbs) caused a marked downregulation of COX-2. To assess the impact of COX-2 inhibition on the efficacy of anti-CAIX CAR T cells, we observed that CAR T cell therapy alone induced approximately 65% tumor cell death. However, when combined with celecoxib, the cytotoxic effect decreased to ~35%, indicating that COX-2 inhibition may impair the antitumor activity of CAIX-directed CAR T cells. Our findings indicate a regulatory interplay between CAIX and COX-2 in ccRCC. Targeting these pathways individually may provide antitumor benefits; however, their combined modulation could negatively impact the efficacy of CAIX-directed immunotherapies such as CAR T cells. Supported by FAPESP (2018/17656-0).

Keywords: CAIX immunotherapy;CAR T cells;COX-2 inhibition.

3044 - Category: TUMOR IMMUNOLOGY (TU)

Effect of palmitic acid and omega-3 in carcinogenic and lipid metabolism of pancreatic adenocarcinoma cells

PAIVA, R.B.L.; MAIA, N.S.R.; CALIXTO, G.F.G.; BEZERRA, S.P.; MAGALHÃES, K.G.. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

INTRODUCTION: Among other omega-3, docosahexaenoic acid (DHA) has been studied to prevent and treat several diseases, including cancer. Palmitic acid (PA) is the most common unsaturated fatty acid, usually related to a worst prognosis in cancers. Pancreatic adenocarcinoma is an aggressive malignancy and is responsible for most of pancreas cancer-related deaths.

METHODS: Mia-Paca-2 cells were stimulated or not with DHA or PA at different times and concentrations. Cell viability was assessed by MTT assay and analyzed by spectrophotometry. Membrane pore formation was assessed by propidium iodide uptake and analyzed by fluorescence spectrophotometry and by LDH release assay analyzed by spectrophotometry. Cell proliferation was analyzed by CFSE probe and cytometry. Oxygen reactive species production was measured by DCFDA probe and analyzed by spectrophotometry and cytometry. Lipid droplet biogenesis was analyzed by bodipy and cytometry or by Oil red with hematoxylin staining and analyzed by microscopy.

RESULTS: DHA, but not PA, was able to reduce Mia paca-2 cells viability in a time and dose dependent way. Neither DHA or PA were able to induce LDH release nor induce membrane pore formation. Oxygen reactive species production was induced by DHA at 8 hours and also at 24 hours. The utilization of n acetyl cysteine inhibited DHA induced cell death. DHA reduced cell proliferation. PA was able to induce lipid droplet accumulation at 48 and 72 hours at a higher concentration. Both DHA and PA increased the number of lipid droplets at 24 48 and 72 hours.

CONCLUSION: Our data suggest that omega-3 DHA but not PA can reduce cell viability of pancreatic cancer cells, by the induction of a non-lithic cell death. In addition, omega-3 DHA and PA had opposite effects on lipid droplet biogenesis. Taken together, our results suggest there is an antagonism effect of omega-3 DHA and PA in pancreatic cancer cells, demonstrating the potential antineoplastic of omega-3 DHA against this cancer.

Keywords: Fatty-acids;Pancreatic cancer;Lipid droplet.

3045 - Category: TUMOR IMMUNOLOGY (TU)

Extracellular Vesicles from Cervical Cancer Cell Lines Modulate Human Monocyte Activation

INOUE, M.G.S.¹; LASSO, J.A.L.¹; ANDRADE, L.N.D.S.²; LEPIQUE, A.P.¹. 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. FACULDADE DE MEDICINA DA USP, SÃO PAULO - SP - BRASIL.

Cervical cancer (CC) remains a significant health burden worldwide, particularly in developing countries. CC development depends on tolerogenic mechanisms, including towards Human Papillomavirus (HPV), its main etiology. Tumor-derived extracellular vesicles (EVs) have been shown to play a role in immune evasion and progression in several types of cancer. HPV transformed cells change EVs amount, size and cargo. Therefore, we raised the hypothesis that EVs isolated from cervical cancer cell lines could modulate immune responses. Our objective is to investigate cervical cancer cell lines derived EVs immunomodulatory effects on monocytes isolated from healthy donor PBMCs. EVs were isolated from HeLa, SiHa and C33A cells, by ultracentrifugation of culture supernatants and characterized by nanoparticle tracking analysis. Monocytes were immunophenotyped by flow cytometry. After CD14⁺ cell sorting, monocytes were treated with lipopolysaccharide (LPS), EVs or a combination of both for 3 days. EVs alone had limited effects on monocytes. However, in LPS-activated monocytes, EVs significantly decreased the percentage of HLA-DR⁺ cells, as well as inhibited CD80, CD163, and CD206 expression. These findings suggest that cervical cancer-derived EVs interfere with monocyte activation in the presence of inflammatory stimuli, possibly impairing classical activation and skewing their differentiation. This result suggests that EVs may be part of the tolerogenic mechanisms triggered by cervical cancer.

Keywords: Extracellular vesicles;Monocytes;Cervical Cancer.

3046 - Category: TUMOR IMMUNOLOGY (TU)

Semaglutide on breast cancer evolution and progression in a murine model

CHAVES, T.C.; COSTA, J.C.; CYPRESTE, L.T.; RAMOS, P.E.R.; GUARNEIRE, G.J.; CARLI, A.D.P.; ALVES, C.C.D.S.; DE CASTRO, S.B.R.. UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI, TEÓFILO OTONI - MG - BRASIL.

Introduction: Breast cancer remains a major public health concern, with increasing incidence and limited treatment options for aggressive subtypes. Semaglutide is a GLP-1 receptor agonist approved for type 2 diabetes and is also used to control body weight. Semaglutide has been used by women of different ages, with an unknown role in several diseases, such as breast cancer. **Objectives:** This study aimed to evaluate *in vitro* and *in vivo* the effects of semaglutide on breast cancer development and progression in individuals exposed to semaglutide. **Methodology:** MCF-7, MDA-MB-231 and 4T1 cells were cultured in 96-well plates at 2×10^5 cells·mL⁻¹ in supplemented RPMI-1640, and maintained at 37 °C in 5% CO₂. Cells were maintained for 48h in the presence or absence of semaglutide (10, 100, 1000, and 10.000 nM). Doxorubicin was used as a cell death control. Cellular viability was measured by MTT assay. *In vivo*, breast cancer was induced with the application of 4T1 in the mammary gland of female BALB/c mice. The mice were divided into four groups (n=8 mice/group). Three groups were induced with breast cancer, and the negative control group was not induced and was untreated. The induced groups were treated with semaglutide (s.c. 60 µg/kg, daily), doxorubicin (i.p 5mg/kg, once a week), or untreated. Semaglutide treatment was performed 7 days before induction and maintained for 31 days after cancer induction. Tumor growth was monitored for 31 days. **Results:** *In vitro*, semaglutide maintained cell viability at values above 70%. *In vivo*, a difference was observed in tumor growth on the 31st day after induction in the group treated with semaglutide compared to the induced-untreated group (299.01±34.39 versus 366.27±43.42). **Conclusions:** Although no difference in tumor development was initially observed, on the 31st day after induction, a reduction in tumor size was observed. Therefore, a longer follow up period is necessary to verify the role of semaglutide in tumor development.

Keywords: GLP-1 RAs; cytotoxicity; triple-negative breast cancer.

3058 - Category: TUMOR IMMUNOLOGY (TU)

Identification of immune checkpoints markers in HPV-associated cervical lesions

LOPES, G.D.S.¹; DE ANDRADE, C.V.²; DE MELO, Y.L.M.F.¹; RUSSOMANO, F.B.²; DE LIMA, E.H.R.¹; BONECINI, G.¹; FERNANDES, A.T.G.¹. 1. INI/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. IFF/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Cervical cancer (CC) is the fourth most common type of cancer among women and the third cause of cancer-related death in women worldwide. The association between CC and Human Papillomavirus (HPV) has been described. HPV-infected cells may evade immune surveillance through several mechanisms, including the expression of the programmed cell death protein (PD-1) and its ligand (PD-L1), which are involved in regulating T cell activation. PD-1/PD-L1 is recognized as a key immune evasion mechanism expressed in lymphocytes, macrophages, and dendritic cells. The aim of this study is to evaluate the expression of markers such as CD8, CD4, PD-1, PD-L1, and TNF-α in intraepithelial (IE) and marginal stroma (MS) from cervical intraepithelial neoplasia (CIN) I, II, III, CC and control groups by immunohistochemistry. This study included 100 women, with 20 in each group evaluated. In relation to IE area, no statistical differences were found in any of the studied markers. However, in the MS area, increased CD8⁺ cells distributions in the CIN II group and of PD-1⁺ and PD-L1⁺ cells in the CC group were observed when compared to the control group. An increased distributions of TNF-α⁺ cells in the CIN II group were observed when compared to CIN I group. Furthermore, positive correlations were observed between PD-1⁺ and TNF-α⁺ cells and between CD4⁺ and TNF-α⁺ cells in the MS of the CIN I group; between PD-1⁺ and CD4⁺ cells and between CD4⁺ and TNF-α⁺ cells in the MS of the CIN II group; between PD-1⁺ and CD4⁺ cells in the MS of the CIN III group; and between PD-1⁺ and TNF-α⁺ cells in the IE of the CIN III group. In the immunoscore evaluation, an increase in the 'high' profile was observed in the CIN II group compared to the CIN I group, and in the CC group compared to CIN I, in the CD8⁺/TNF-α⁺, CD4⁺/TNF-α⁺, CD8⁺/CD4⁺/TNF-α⁺, and PD-1⁺/CD4⁺/TNF-α⁺ profiles. Our results suggest an association between these markers and the development of cervical lesions.

Keywords: Cervical lesions; HPV; immune checkpoints.

3069 - Category: TUMOR IMMUNOLOGY (TU)

Investigating the effects of Metformin isolated or associated with the Omega-3 DHA on the carcinogenic parameters of human ovarian cancer cells in vitro

DA COSTA, L.B.; DE ARAÚJO, M.N.V.; DO NASCIMENTO, G.P.; BEZERRA, S.P.; MAGALHÃES, K.G.. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

Cancer is a group of chronic diseases characterized by abnormal cells that resist cell death, proliferate in a disordered manner, and invade tissues. Ovarian cancer is a highly lethal disease due to its frequent diagnosis at late stages. Metformin (MET) and omega-3 DHA are relevant compounds due to their antitumor effects in various malignancies, as they modulate the response to chemotherapy and reduce tumor aggressiveness. However, the combined potential of these two compounds remains unexplored. In this context, the objective of our study was to evaluate the effects of MET and DHA, alone or in combination, on carcinogenic parameters in the human ovarian cancer cell line A2780. A2780 cells were treated with different doses of MET and DHA, either separately or in combination. Cytotoxicity was assessed using the colorimetric MTT assay to measure cell viability based on mitochondrial activity. To evaluate cell proliferation, cells were stained with the CFSE probe and analyzed by flow cytometry. Oxidative status was assessed using the fluorescent probe DCF-DA in a 0–6 h time course. Membrane pore formation was evaluated by staining cells with propidium iodide through 0–12 h time course. Our data show a significant reduction in cell viability after treatment. Cell proliferation analysis revealed that MET did not impact proliferation; however, treatment with DHA and the combined therapy significantly reduced proliferation. Kinetic analysis using DCF-DA suggests that metformin does not exert an oxidative effect during early time points, while co-treatment induces reactive oxygen species (ROS) production at 3 h when compared to DHA and MET separately. Finally, membrane pore formation was mainly observed from 9 h onward in the DHA condition and, to a lesser extent, in the co-treatment. Our results suggest that MET and DHA, separately or in combination, reduce cell proliferation and viability and modulate oxidative status and membrane pore formation in A2780 cells.

Keywords: Ovarian cancer ;Metformin;Omega-3 DHA.

3082 - Category: TUMOR IMMUNOLOGY (TU)

AR33, a resveratrol analogue, affects viability, proliferation, and migration of murine melanoma cells.

PEREIRA, L.G.L.; ALMEIDA, A.P.G.; GUEDES, M.C.M.R.; DE OLIVEIRA, L.G.; REIS, M.O.; SEGHE TO, J.A.; PASCHOALIM, M.E.D.O.; MEINEL, R.S.; DA SILVA, A.D.; MACEDO, G.C.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Introduction: Melanoma is an aggressive malignant neoplasm with high metastatic potential. Although several therapies are currently available, they show significant limitations, reinforcing the need for novel therapeutic agents. In this context, resveratrol (RVT), a natural polyphenol, has demonstrated antitumor and immunomodulatory activities, but its in vivo application is restricted by low solubility and bioavailability. The development of RVT analogues, such as AR33, represents a promising strategy to overcome these limitations and to potentially modulate immune responses within the tumor microenvironment. This study aimed to investigate the antitumor potential of AR33 in melanoma.

Methods and Results: The cytotoxicity of AR33 was assessed by MTT assay in B16F10 (murine melanoma), L929 (murine fibroblast), and HaCaT (human keratinocyte) cell lines. In B16F10 cells, clonogenic and CFSE assays were performed to evaluate effects on colony formation and proliferation, respectively, while a wound-healing assay assessed cell migration. As results, at 50 μ M, AR33 reduced B16F10 cell viability to below 60%, with acceptable cytotoxicity in non-tumorigenic cells. AR33 markedly inhibited colony formation, showing superior efficacy to RVT. Proliferation assays demonstrated preservation of CFSE fluorescence over time, consistent with an antiproliferative effect. In addition, AR33 inhibited melanoma cell migration more effectively than RVT. **Conclusion:** These findings indicate that AR33 has promising antitumor activity, surpassing RVT in multiple functional assays. Considering its previously described anti-inflammatory properties, ongoing studies will investigate whether AR33 also modulates immune cell subsets, such as dendritic cells, macrophages, and T lymphocytes, within the tumor microenvironment, thereby contributing to its antitumor efficacy. **Financial support:** FAPEMIG (RED 00067-23; RED 00096-22; APQ 02177-22).

Keywords: Melanoma;Analogue;Resveratrol.

3108 - Category: TUMOR IMMUNOLOGY (TU)

Generation of anti-GD2 CAR-T Cells by sleeping beauty transposon system

VIEIRA, L.M.¹; ARAGAO, E.A.A.¹; DE LIMA, L.N.¹; MACHADO, S.A.²; CORREIA, E.M.¹; BONAMINO, M.H.¹. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

Immunotherapy with T cells modified with Chimeric Antigen Receptors (CARs) is one of the most promising antitumor strategies. The CAR gene can be integrated into the genome using the Sleeping Beauty system, which employs two vectors: one carrying the CAR gene within the transposon, and another encoding the transposase that mediates genomic integration. Some solid tumors, such as retinoblastoma, neuroblastoma, osteosarcoma, melanoma and glioblastoma, are known to commonly express the ganglioside GD2, making it good target for CAR-T cell immunotherapy. The aim of this work was to synthesize and validate an anti-GD2 CAR plasmid (14G2A clone) and to generate CAR-T cells from peripheral blood mononuclear cells (PBMCs). 20 µg of the anti-GD2 CAR plasmid and 1 µg of the SB transposase plasmid were electroporated into 3×10^7 PBMCs pre-activated for 48 hours. Cells were then analyzed for up to 8 days post-electroporation. Additionally, CAR-T cells were co-cultured with 3×10^4 T-98G glioblastoma cells at effector-to-target (E:T) ratios of 5:1 and 1:1. At 24, 48, and 72 hours post co-culture, adherent tumor cells were stained with crystal violet, and absorbance was measured at 592 nm. An average frequency ($n = 7$) of CAR-T cells positive for anti-GD2 was 7.63%, 17.9%, and 16.1% on days 1, 6, and 8 of expansion, respectively. After 72 hours of co-culture, the anti-GD2 CAR-T (14G2A) group reduced absorbance by 76.3% and 74.5% at the 5:1 ratio, and by 59.1% and 74.3% at the 1:1 ratio, compared to the T-98G alone group and the MOCK group (PBMCs electroporated without the CAR plasmid), respectively. These results demonstrate stable expression of the anti-GD2 CAR plasmid (14G2A) in PBMCs, following electroporation using the Sleeping Beauty system, and in vitro antitumor activity. Future steps in this project will include additional functional assays, such as in vivo validations.

Keywords: Immunotherapy; CAR-T cells; Ganglioside GD2.

3126 - Category: TUMOR IMMUNOLOGY (TU)

Oxidative and apoptotic effects of Venetoclax and JQ1 combination in acute myeloid leukemia cell lines

KIMMEMGS, T.N.¹; HUMMEL, G.A.²; MERCURI, R.L.V.²; MENDONÇA, G.D.S.²; LEOCATA, B.²; PANSONATO, .L.²; .CAMARA, N.O.³; GALANTE, P.A.F.²; AMANO, M.T.². 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. INSTITUTO DE ENSINO E PESQUISA HOSPITAL SÍRIO-LIBANÊS, SÃO PAULO - SP - BRASIL; 3. INSTITUTO DE CIÊNCIAS BIOMÉDICAS DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Acute myeloid leukemia (AML) is known to be a heterogeneous and severe hematologic cancer. The BCL-2 inhibitor, Venetoclax (VEN), is an established treatment indicated for patients ineligible for chemotherapy, but new therapies are being sought for patients unresponsive to VEN. AML presents high oxidative stress and elevated production of reactive oxygen species (ROS), although its precise role remains unclear. The BRD4 protein, an epigenetic regulator of the BET family, can control the expression of several genes, including genes linked to oxidative homeostasis. Studies indicate that its inhibition can affect redox balance. Thus, we sought to investigate the effect of the combination of JQ1 (BRD4 inhibitor) and VEN on oxidative homeostasis in AML cell lines. Based on the IC₅₀ obtained by dose-response assay, HL-60 and MOLM-13 cell lines were more sensitive to VEN than HEL and U937. Combined treatment reduced cell proliferation in all cell lines. The combination also altered the expression of genes associated with the antioxidant response (*JUN*, *FOS*, and *HO-1*), and JQ1 reduced ROS production in all cell lines evaluated. The combination of inhibitors also altered the expression of genes linked to the apoptosis pathway (*MCL-1*, *BIM*, *PUMA*, and *NOXA*), in addition to increasing the cell death in three of the four cell lines analyzed. We identified the nuclear presence of HO-1, indicating a possible non-canonical function of this protein. Finally, RNA-Seq analysis in the HL-60 and U937 cell lines showed a basal difference between genes involved in the antioxidant response and cell death, suggesting that distinct molecular profiles may influence treatment sensitivity. These findings indicate that the combination of VEN and JQ1 may affect oxidative homeostasis and the apoptotic profile of different AML cell lines. Furthermore, the presence of nuclear HO-1 suggests atypical functions of this protein, highlighting new possibilities for further research.

Keywords: Oxidative Stress; BET inhibitors; Venetoclax.

3128 - Category: TUMOR IMMUNOLOGY (TU)

Investigation of LC3-associated phagocytosis (LAP) in tumor progression and immunotherapy resistance in solid tumorsSOUZA, W.C.D.; TERREL, D.L.A.; CUNHA, L.D.. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

The composition of the microenvironment in solid tumors directly influences cancer progression and response to therapies. Among the cells present in this ecosystem, tumor-associated macrophages (TAMs) represent the most abundant and heterogeneous population within the immune compartment. In this context, the classical M1/M2 polarization dichotomy is insufficient to describe the functional diversity of TAMs, and there has been a recent increase in the characterization of markers to identify subpopulations based on their association with prognosis and therapeutic resistance. However, these markers still require a deeper understanding of their origin and biological relevance in these cells. In this study, we propose to investigate how the LC3-associated phagocytosis pathway (LAP) influences the functional diversity of TAMs during the clearance of dead tumor cells (efferocytosis). Our focus is to understand the relationship between this pathway and newly identified markers associated with poor prognosis. To this end, we will use murine models with deletion of LAP components and breast cancer as the solid tumor model of interest. We aim to determine how LAP impacts tumor progression in cancers with different degrees of immune exclusion [hormone receptor-positive (HR+) vs. triple-negative breast cancer (TNBC)] and the composition of their immune microenvironment (TAMs and tumor-infiltrating lymphocytes – TILs). Based on these findings, we seek to understand how LAP influences the response to immune checkpoint blockade therapy. Investigating the role of LAP in shaping the heterogeneity of pro-tumoral macrophages and the composition of the immune niche may reveal new targets to enhance the efficacy of immunotherapy in solid tumors.

Keywords: Tumor-associated macrophages; efferocytosis; non-canonical autophagy.

3133 - Category: TUMOR IMMUNOLOGY (TU)

Molecular Profiling of the Tumor Microenvironment in Thyroid Cancer via Real-Time Mass Spectrometry

SILVA, G.G.¹; CERNEA, C.R.²; POTENZA, A.D.S.²; PEREIRA, F.K.F.¹; DE FIGUEIREDO, A.B.¹; FERREIRA, C.E.D.S.²; EBERLIN, L.S.³; GOLLOB, K.J.¹. 1. INSTITUTO ISRAELITA DE ENSINO E PESQUISA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 3. BAYLOR COLLEGE OF MEDICINE, HOUSTON - ESTADOS UNIDOS DA AMERICA.

Understanding the tumor microenvironment is central to advancing tumor immunology, as immune responses are shaped by the spatial and biochemical landscape of the tumor and its surrounding stroma. In thyroid cancer, characterizing this complex microenvironment can reveal metabolic and immunoregulatory mechanisms that influence tumor progression. Mass spectrometry-based approaches are powerful tools for identifying molecules involved in immune modulation, but conventional techniques are limited by sample preparation time and loss of spatial resolution. The MasSpec Pen is an innovative device that enables rapid, minimally invasive molecular analysis of fresh tissues using a gentle aqueous extraction, preserving tissue integrity. This study applied the MasSpec Pen to thyroid tumor specimens intraoperatively, aiming to distinguish tumor from adjacent non-tumoral tissues and to explore region-specific molecular signatures relevant to immune regulation. Samples were analysed immediately after surgical excision using an Orbitrap Exploris 120 mass spectrometer in full scan mode (130-1800m/z, 120,000 resolution, negative ion mode). A 3 mm pen tip was used with a five-second sampling time and ten-second aspiration step. Spectra were collected in triplicate from tumor and healthy regions. The data revealed distinct molecular profiles between tissue types, including differential abundance of lipids and small metabolites. High mass accuracy enabled tentative identification of biologically relevant compounds such as ceramides, fatty acids, and phospholipids-molecules known to influence immune cell recruitment, activation, and suppression. These findings demonstrate the MasSpec Pen's potential to support tumor immunology studies by rapidly mapping the biochemical features of the TME in situ. This study was financed by FAPESP (2023/16341-3). This study was approved by the Ethics Committee of the Hospital Israelita Albert Einstein (CAAE: 77118923.0.0000.0071).

Keywords: Tumor Microenvironment; Mass Spectrometry; Thyroid Cancer.

3151 - Category: TUMOR IMMUNOLOGY (TU)

Cytokine levels and their association with the risk of precursor B-cell lymphoblastic leukemia.

CABRAL, Y.D.S.¹; DA CRUZ, D.A.M.²; GRANADO, E.T.³; AVILA, E.P.N.³; POMBO-DE-OLIVEIRA, M.S.⁴. 1. IOC, RIO DE JANEIRO - RJ - BRASIL; 2. LABORATORY ON THYMUS RESEARCH, OSWALDO CRUZ INSTITUTE/FIOCRUZ, RIO DE JANEIRO/RJ, BRAZIL, RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTE GONÇALO MONIZ, OSWALDO CRUZ FOUNDATION, FIOCRUZ, BAHIA, BRAZIL, SALVADOR - BA - BRASIL; 4. NACIONAL CANCER INSTITUTE, INCA, RIO DE JANEIRO/RJ, BRAZIL, RIO DE JANEIRO - RJ - BRASIL.

Precursor B-cell acute lymphoblastic leukemia (B-ALL) is the most prevalent subtype of pediatric neoplasms, with a high incidence rate between 2 and 5 years of age. The causal mechanisms involve both genetic predispositions and environmental exposures. The initial genetic alterations typically occur in utero, leading to the emergence of a somatic clonal mutation, with the immune system playing a crucial role in the proliferation of this initial clone. Immune dysregulation at birth, including significantly lower neonatal concentrations of sIL6R α , IL-8, IL-10, IL-12, and TGF β 1, as well as elevated levels of IL-6, IL-4, IL-17, and IL-18, suggest a connection between immune profile and susceptibility to B-ALL. The aim of our study was to investigate the innate immune profile of Brazilian children diagnosed with B-ALL and to estimate the magnitude of associated risk factors. A cohort of 254 incident B-ALL cases diagnosed between 2017 and 2020 was included in this study. From this cohort, 88 samples were selected for detailed analysis of blast populations and T, B, and NK cell subpopulations. A multiplex assay (CBA - BD kit for humans) was employed for cytokine quantification following short-term cell culture (24 and 48 hours) in appropriate media. Concentrations of IFN- γ , TNF- α , IL-17, IL-4, IL-6, and IL-2 were measured using flow cytometry. Statistical analyses included Z-tests to evaluate the randomness of sample selection and Kruskal-Wallis tests for interleukin comparisons. Among the cases, 82 were classified as B-ALL and 6 as T-ALL. A twofold increase in IFN- γ production was observed in infants compared to older children. IFN- γ expression was approximately ten times higher than that observed in cord blood (CB). IL-10 expression was reduced by about 50% in comparison to CB, and IL-6 expression showed a nearly tenfold decrease. Additionally, a significant association was found between race/ethnicity and leukemia risk.

Keywords: Leukemia ;B-cell;Immune system.

3186 - Category: TUMOR IMMUNOLOGY (TU)

Glioblastoma-irradiated cells differentially modulate the polarization of tumor-associated neutrophils

GENTIL, G.F.D.S.; MARTINS, S.D.F.; LOPES, L.B.; OLIVEIRA, G.M.; REITER, K.C.; TROMBINI, H.; BRAGANHOL, E.. UFCSA, PORTO ALEGRE - RS - BRASIL.

Glioblastoma (GB) is the most aggressive tumor of the central nervous system. Standard treatment includes radiotherapy (RT), but GB frequently recurs due to its ability to resist therapies. RT initially creates a pro-inflammatory tumor environment by recruiting immune cells such as neutrophils (N0). However, tumor cells can later modulate N0 into a pro-tumoral phenotype, potentially contributing to radioresistance and recurrence. In this study we aimed to investigate the impact of tumor-irradiated cells (GB-RT) on N0 phenotype. Non-irradiated (non-GB-RT;U138 cell line) and GB-RT (5Gy) were cocultured with freshly isolated human N0 for 24 h. The N0 phenotype was analyzed by flow cytometry using the markers CD11b, CD170, HLA-DR, LOX-1, PDL-1, CD39, and CD73. N0 cultured alone were used as negative control, and N0 stimulated with Interferon- γ (INF- γ) and TGF- β were used as positive controls of N1 and N2 polarization, respectively. Data analysis was performed using FlowJo 10 and clusters were generated using the X-shift plugin. In total, X-shift identified 29 clusters of N0 based on differential marker expression. We found 2 clusters differently expressed between N0 cocultured with non-GB-RT and GB-RT cells. Indeed, clusters 15 and 25 were predominantly present in N0 exposed to non-GB-RT and GB-RT cells, respectively ($p \leq 0.01$; $p \leq 0.05$) and TGF- β and INF- γ did not differ significantly between them. Marker analysis revealed that cluster 15 was characterized as CD11bhi, CD170hi, LOX-1+, PDL-1low, HLA-DRlow, CD39low and CD73hi, whereas cluster 25 was characterized as CD11bhi, CD170hi, LOX-1+, PDL-1low, HLA-DRlow, CD39low and CD73low. In conclusion, these two clusters mainly differ in CD73 expression. CD73 plays an important role in adenosine production and immunosuppressive environment. Therefore, N0 exposed to GB-RT cells exhibits a reduced CD73hi population, suggesting a pro-inflammatory phenotype 24 h after RT.

Keywords: Glioblastoma;Neutrophils;Radiotherapy.

3226 - Category: TUMOR IMMUNOLOGY (TU)

Cell fusion strategies for the generation of hybrid immune cells with enhanced antitumoral effects

DA SILVA, L.H.R.; PEREIRA, V.A.C.; LIMA, M.P.O.; ROCHA, V.G.; COUTO, S.C.F.; RAMOS, R.N.. LABORATÓRIO INVESTIGAÇÃO MÉDICA EM PATOGÊNESE E TERAPIA DIRIGIDA EM ONCO-IMUNO-HEMATOLOGIA (LIM31), FACULDADE DE MEDICINA DA USP, SÃO PAULO - SP - BRASIL.

Growing evidence suggests that cellular hybrids do not merely represent a phenotypic combination of two parental cells but rather exhibit a unique gene expression profile with specific functional features. We aim to generate hybrids between immune cells, seeking to produce hybrid phenotypes that combine cellular functions to improve anticancer immunotherapeutics. This strategy is interesting because leucocytes are an essential part of cancer biology, in a way that inducing cell fusion could lead to enhanced response by complementing cell phenotypes and directing additive effects. To establish a cell fusion protocol, we accessed polyethylene glycol (PEG)- and electrofusion-mediated methodologies using Nalm-6 cells, evaluating technical parameters such as protocol feasibility, cell viability, and fusion efficiency. All tested PEG-mediated protocols preserved cell viability ($>89,4\% \pm 10,6\%$). Compared to mixture, the relative frequencies of fused cells were: 0.37% (protocol 1), 3.41% (protocol 2) and 1.57% (protocol 3) via flow cytometry analysis. Using confocal microscopy, we noted that all the protocols generated typical hybrid cells, evidenced by double nuclei and fused cell membrane. On the other hand, electrofusion compromised cell viability, which limited the accurate assessment of fusion frequency. Efficiencies for the electrofusion protocols 1, 2, and 3 were 6.74%, 9.27%, and 12.29%, respectively, but with the predominance of non-viable cells. Both PEG- and electrofusion-mediated methodologies presented comparable feasibility, although they differ in costs, control of the process and cell yield. This study validates distinct PEG- and electroporation-mediated fusion protocols as a previous step for immune hybrid generation. Considering the lack of a systematic comparison of these artificial cell fusion technologies in literature, these standardization findings provide a more comprehensive framework for exploration of cell fusion in broader immunologic applications

Keywords: Cell Fusion;PEG;Electrofusion.

3236 - Category: TUMOR IMMUNOLOGY (TU)

TARGETING TUMOR-ASSOCIATED MACROPHAGES WITH CAR-T CELLS: A NOVEL APPROACH FOR SOLID TUMORS

PEREIRA, V.A.C.; LIMA, M.P.O.; DA SILVA, L.H.R.; DE CASTRO, L.F.; MATSUMURA, E.L.M.; ALMEIDA, R.R.; COUTO, S.C.F.; BARROS, L.R.C.; RAMOS, R.N.; ROCHA, V.G.. LABORATÓRIO INVESTIGAÇÃO MÉDICA EM PATOGÊNESE E TERAPIA DIRIGIDA EM ONCO-IMUNO-HEMATOLOGIA (LIM31), FACULDADE DE MEDICINA DA USP, SÃO PAULO - SP - BRASIL.

Tumor-Associated Macrophages (TAMs) are abundant cells in the tumor microenvironment and have the ability to suppress T lymphocytes. Among distinct subsets, the CD163⁺ TAMs are classically associated with poor prognosis in distinct tumor types including breast, ovarian and pancreatic cancers. Chimeric Antigen Receptor T cells (CAR-T cells) have revolutionized the treatment of hematological cancers, however, their success in solid tumors is limited by intratumoral immunosuppression. Therefore, strategies aimed at eliminating TAMs may show promising therapeutic potential. Thus, using a lentiviral vector, our study proposes the use of a second-generation CAR-T cell against CD163 (α CD163) aiming to eliminate CD163⁺ TAMs. We compared α CD19 CAR-T and α CD163 CAR-T cells transduction and functional status using either a lentiviral or a piggyBac transposase system. CAR transduction was performed in T lymphocytes 24 hours after activation, showing an efficiency of about 39% ($\pm 1,34\%$, $n=2$) for α CD19 CAR and about 10.3% ($n=1$) of α CD163 CAR expression. CAR-T cells showed up-regulation of memory and activation markers (CCR7, CD45RO and HLA-DR) 10 days post-transduction. Furthermore, the expression levels of memory markers CCR7⁺CD45RO⁺ and activation marker HLA-DR were similar when comparing non-transduced T cells with all transduced lymphocytes. Cytotoxic activity of α CD19 CAR-T was tested against THP-1 cells and CD19⁺ cell lines. Results show a dose-dependent cytotoxic response across the 3 tested donors using the THP-1 cell line. Also, a higher CD19⁺ NALM6 cell killing was promoted by α CD19 CAR-T when compared to non-transduced counterparts. These findings support the antitumor potential of expanded CAR-T cells, marked by an enhanced cytotoxic function. Future steps involve producing α CD163 CAR-T cells and evaluating cytotoxicity against CD163⁺ myeloid cells *in vitro*.

Keywords: Immunotherapy;CAR-T cells;Tumor-Associated Macrophages.

3274 - Category: TUMOR IMMUNOLOGY (TU)

The role of fibroblasts and their soluble factors in the growth of murine breast cancer spheroids

DA CUNHA, F.N.; MASCARENHAS, G.D.; WERNECK, M.B.D.F.; DE MELO, C.B.; DIAZ, B.L.. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

The tumor microenvironment (TME) plays a crucial role in cancer progression, with soluble mediators and stromal cells acting as key modulators of cancer cell behavior and responses. To investigate the role of fibroblasts in the TME of breast cancer, we established a three-dimensional model using homotypic and heterotypic spheroids composed of NIH/3T3 fibroblasts and two murine breast cancer cell lines with distinct metastatic behavior: 4T1 and 67NR. In homotypic spheroids assembled with different initial cell numbers, both cell lines exhibited linear and modest growth over 7 days. The growth rates of 4T1 spheroids were 0.0059 mm²/day (125 cells) and 0.0054 mm²/day (500 cells). The 67NR spheroids grew faster (125 cells: 0.00715 mm²/day; 500 cells: 0.0120 mm²/day). In contrast, NIH/3T3 spheroids decreased in size over time (−0.0054 and −0.0108 mm²/day for 2,375 and 9,500 cells, respectively) and produced increased levels of IL-6 in a behavior reminiscent of senescence. When cells were combined in a fixed proportion of 500:9,500 cancer cell:fibroblast, in heterotypic spheroids, we observed an increased growth rate for 4T1 + NIH/3T3 spheroids (0.3415 mm²/day) when compared with homotypic spheroids. Meanwhile, 67NR + NIH/3T3 spheroids showed only marginal growth over 7 days of culture. To address if soluble mediators could explain the growth behavior of heterotypic spheroids, we exposed homotypic spheroids with conditioned medium (CM) from the complementary cell line. While NIH/3T3-CM reduced tumor growth in both cancer cell lines, 4T1-CM induced a substantial increase in fibroblast only spheroids area from D3 to D7 (0.144 to 0.207 mm²; with 9,500 NIH/3T3), while 67NR-CM had no effect. We conclude that paracrine and juxtacrine signaling exist between breast cancer cells and fibroblasts, and this response is dependent on the characteristics of the cancer cell. The influence of these signals on the hematopoietic component of the TME is currently under investigation.

Keywords: Breast cancer;Fibroblasts;Tumor microenvironment.

3287 - Category: TUMOR IMMUNOLOGY (TU)

Soluble Factors from Obese Adipose Tissue Outperform Small and Large Extracellular Vesicles in Promoting Tumor Progression through Triple-Negative Breast Cancer Cell Migration and Inflammatory Cytokine Release

SEIBERLICK, O.S.G.; VENERANDO, L.A.F.; JANUZZI, L.L.R.; SEIBERLICK, T.D.S.O.; LIMA, M.J.T.; SOARES, S.M.A.; HOTTZ, E.D.; DIB, P.R.B.; CARVALHO, B.D.O.; GAMEIRO, J.. UFJF, JUIZ DE FORA - MG - BRASIL.

Obesity significantly increases breast cancer risk through chronic dysfunction of adipose tissue (AT), which reshapes the tumor microenvironment. However, the pathways by which vesicular and soluble mediators orchestrate this dialogue remain poorly elucidated. In the present study, we explored the differential impact of distinct AT-derived extracellular vesicle (EV) fractions, as well as soluble factors, on triple-negative breast cancer (TNBC) progression. Small EVs (sEVs), large EVs (IEVs), and EV-depleted conditioned medium (CM) were isolated from visceral AT of bariatric-surgery patients (n=6, mean BMI 44.1 kg m⁻²; three males, three females) and initially characterized by dynamic light scattering and BCA assay. TNBC MDA-MB-231 cells were exposed to sEVs, IEVs, or CM (25 µg mL⁻¹ protein) and analysed for migration (wound-healing assay) and for cytokine release by (enzyme-linked immunosorbent assay, ELISA). Unexpectedly, CM induced the greatest wound closure, achieving a 21.3% gap reduction, while IEVs presented 18.0% after 48h, compared to only 4.3% for sEVs. Notably, IEVs from class-I obesity donors induced higher migration than those from class-III obesity. Furthermore, only CM selectively elevated interleukin-8 (IL-8, 142.1 pg mL⁻¹; p=0.002) and interleukin-6 (IL-6) levels, while interleukin-1β (IL-1β) remained minimal, revealing distinct inflammatory signatures within the soluble secretome of obese AT. Another finding, basal CM contained high levels of IL-6 (527.4 pg mL⁻¹; p=0.0017), which decreased by 72% (81.1 pg mL⁻¹) after MDA-MB-231 exposure, suggesting cellular uptake of this cytokine. Our results highlight the complexity and complementarity of EV- and secretome-mediated intercellular communication, demonstrating that soluble mediators in obese AT surpass extracellular vesicular signals in driving TNBC aggressiveness and positioning the soluble fraction as a potential therapeutic target in obesity-associated TNBC.

Keywords: Triple-negative Breast Cancer;Obesity;Extracellular Vesicles.

3288 - Category: TUMOR IMMUNOLOGY (TU)

Evaluation of a peptide vaccine's immunogenicity and efficacy in a TNBC mouse model

BOBERMIN, D.M.; VIEIRA JUNIOR, J.C.; CLAUBERG, E.C.; MAMIGONIAN, O.; DA ROCHA, E.L.; BAFICA, A.L.B.; MANSUR, D.S.. UFSC, FLORIANÓPOLIS - SC - BRASIL.

Among all types of cancer, breast cancer (BC) is one of the most common malignancies worldwide and, despite advances in treatment, remains the leading cause of cancer deaths in women. BC can be classified into subtypes according to the expression of molecular markers, and those that do not express any of the common markers (ER/PR/HER2) are classified as triple-negative breast cancers (TNBCs). In general, TNBC has a poor prognosis and tends to metastasize. Due to the absence of these markers, patients cannot benefit from standard targeted drugs such as hormones and anti-HER2. Thus, it is essential to develop alternatives.

In this work, we used bioinformatic tools to select three peptides derived from the transcriptome of the 4T1 cell line (a TNBC mouse model) that showed the ability to bind MHC molecules. Two of them are MHC class II ligands and contain embedded sequences with predicted MHC class I affinity, enabling the activation of both CD4+ and CD8+ lymphocytes. These peptides were combined with the adjuvant poly(I:C) and Montanide ISA 51, forming an emulsion. Mice were vaccinated with two doses of this emulsion on days 0 and 14. Seven days after the second dose, animals were euthanized and spleens were collected for recall analysis to evaluate IFN- γ production by T lymphocytes. Results showed that the peptide combination could induce a memory response in part of the vaccinated healthy mice.

To test vaccine efficacy against the tumor, we applied the same two-dose protocol to mice with pre-existing tumors (three days after 4T1 cell inoculation). Tumor growth was measured every 3–4 days using a digital caliper. After 30 days, mice were euthanized, spleens were collected, and tumors were dissected and weighed. Despite immune response heterogeneity, mice producing high IFN- γ levels showed smaller tumors than non-responders. Although further studies are needed, the vaccine has shown potential in controlling tumor growth.

Keywords: BREAST CANCER ;VACCINE;TUMOR.

3289 - Category: TUMOR IMMUNOLOGY (TU)

Expanding precision oncology: a genomic and immunologic framework to understand tumor-immune interactions in lung cancer

DA SILVA, A.B.¹; FROZZA, F.T.B.²; SODER, S.²; NUNES, C.P.²; MARQUES, C.G.D.S.²; CORRÊA, B.²; PEREIRA, G.F.P.D.M.²; CALDEIRA, L.E.N.²; MUHLEN, P.H.F.V.²; BONORINO, C.². 1. UFRGS, PORTO ALEGRE - RS - BRASIL; 2. UFCSPA, PORTO ALEGRE - RS - BRASIL.

Although immune checkpoint blockade has transformed treatment in various cancers, patients harboring oncogenic driver mutations can exhibit limited response. Identifying how particular driver mutations - or combinations thereof - influence immune cell infiltration, antigen presentation, and immune evasion pathways remains a critical unmet need in tumor immunology. A cohort of 235 patients of lung cancer was assembled from the Santa Casa de Misericórdia Hospital Complex (Porto Alegre, Brazil), with tumor samples preserved as formalin-fixed paraffin-embedded (FFPE) tissues. Both RNA and genomic DNA were extracted. Genomic profiling is being performed using the OncoPrint Comprehensive Assay Plus DNA panel (Thermo Fisher Scientific). These data were integrated with transcriptomic profiling and histologic findings and custom bioinformatics pipelines are being employed to correlate genomic alterations with immune signatures. So far, 39 samples have been characterized. Analysis of 500 cancer-related genes, identified mutations in key driver genes: EGFR, KRAS, PIK3CA, and TP53, with additional variants in ERBB2, RB1, PTEN, HRAS, RAD51B, RAD54L, PIK3CA, TERT, and MET. Bulk RNA sequencing revealed initial insights into transcriptomic landscape, with downstream analysis still in progress. This integrated approach may identify genomic-immune signatures predictive of therapy response or resistance, offering novel insights for patient stratification in immunotherapy and broadening access to precision oncology beyond high-resource settings.

Keywords: immunogenomics;lung cancer;driver mutations.

3290 - Category: TUMOR IMMUNOLOGY (TU)

Dual CAR-T Based Strategy Targeting CAIX and PD-L1 for Glioblastoma

DE LIMA, N.C.R.¹; DE CAMPOS, N.S.P.¹; BARBOZA, R.²; FERRARI, M.D.F.R.³; SUAREZ, E.R.⁴. 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, DIADEMA - SP - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. UNIVERSIDADE FEDERAL DO ABC, SANTO ANDRÉ - SP - BRASIL.

Introduction: Glioblastoma (GBM) is the most prevalent malignant brain tumor. Hypoxia, a common feature of GBM, leads to the overexpression of proteins such as carbonic anhydrase IX (CAIX). The tumor microenvironment promotes T cell exhaustion and immune evasion, often through high expression of PD-L1, a key immune checkpoint. A promising therapeutic strategy involves chimeric antigen receptor (CAR) T cells targeting tumor antigens. **Objective:** This study aimed to evaluate the *in vitro* efficacy of CAR Jurkat cells targeting CAIX and secreting anti-PD-L1 antibodies against GBM cells. **Methods:** GBM cell lines U251 and A172 were analyzed for basal expression of CAIX and PD-L1 by flow cytometry. Lentiviruses encoding CARs were produced, titrated, and used to transduce Jurkat cells. Jurkat cells expressing CARs (CAR-J) were expanded and characterized. Cytotoxicity assays were performed using luciferase activity, and cytokine secretion analyses (IL-1 β) were conducted by ELISA after co-culture with GBM U251 cells. **Results:** U251 cells expressed higher CAIX and PD-L1 than A172 cells and were selected for the functional assays. Jurkat cells were efficiently transduced. CAR-J targeting CAIX alone or in combination with PD-L1 blockade both induced cytotoxicity, with superior results from the dual-function CAR-J. At a 1:10 T:E ratio, CAR-J Anti-CAIX/CD28 achieved ~25% cytotoxicity, while the CAR-J secreting anti-PD-L1 reached ~60% and secreted significantly more IL-1 β . **Conclusion:** CAR-J cells targeting CAIX and blocking PD-L1 enhance cytokine secretion and cytotoxicity in CAIX+/PD-L1+ GBM cells. We are expanding this approach for primary CAR T cells. This strategy may offer therapeutic benefits in immunosuppressive tumors, such as GBM.

Keywords: Carbonic Anhydrase IX; Glioblastoma; Chimeric Antigen Receptor.

3292 - Category: TUMOR IMMUNOLOGY (TU)

LACTATE AS A PROGRESSION FACTOR FOR CERVICAL CANCER ASSOCIATED WITH HUMAN PAPILLOMAVIRUS

COLONNA, F.C.; LEPIQUE, A.P.. INSTITUTE OF BIOMEDICAL SCIENCES - USP, SÃO PAULO - SP - BRASIL.

Lactate, commonly produced by *Lactobacillus* spp., maintains a protective vaginal microenvironment against pathogenic anaerobic bacteria. Reduced *Lactobacillus* abundance is associated with a higher risk of acquiring sexually transmitted infections, such as Human Papillomavirus (HPV). Conversely, lactate is also a metabolic byproduct generated by glycolytic tumor cells, transported via monocarboxylate transporters (MCTs), and capable of modulating immune and tumor cell function. It also acts as a signaling molecule through its receptor (GPR81/HCAR1), whose activation promotes cell survival and induces tolerogenic phenotype in dendritic cells (DCs), essential for antitumor immune responses. However, how microbial and/or tumor-derived lactate affects local immune responses during cervical cancer progression, particularly in the context of E6 and E7 oncoproteins expressed by HPV, remains poorly understood. We hypothesize that in HPV-infected cells, lactate contributes to cancer progression. Therefore, we investigated the role of lactate in HPV-induced carcinogenesis. For this purpose, we used immortalized human keratinocytes transduced with E6 and E7 (HaCaT pLXSN E6E7) and a control cell line, HaCaT pLXSN, as well as cervical cancer derived cell lines in neat cultures or co-cultures with peripheral blood mononuclear cells (PBMCs) and with monocyte derived dendritic cells (MO-DCs). Our results demonstrated a pro-tumoral role for lactate, promoting epithelial cell proliferation, p65 NF κ B activation, and increased GPR81 expression in HaCaT E6E7, but not in the control cell line. In PBMCs co-cultured with HaCaT E6E7, lactate increased IL-8, IL-6 and TNF- α secretion, NF κ B targets. In Mo-DCs/tumor cells cocultures, lactate led to PD-L1 increased expression. In conclusion, lactate contributes to cervical carcinogenesis in association with the presence of the HPV oncoproteins E6 and E7, by promoting tumor cell growth and creating an immunosuppressive microenvironment.

Keywords: Cervical Cancer; HPV; lactate.

3293 - Category: TUMOR IMMUNOLOGY (TU)

Immune Component Identification and Spatial Organization via IBEX

MORAIS, K.L.P.¹; MARIANO, R.M.D.S.¹; SCARINI, J.F.¹; NUNES, C.M.D.C.¹; SILVA, G.G.¹; ALOIA, T.P.A.²; CORTES, L.G.F.¹; FIGUEIREDO, A.B.¹; DUTRA, W.³; GOLLOB, K.J.¹. 1. CENTER FOR RESEARCH IN IMMUNO-ONCOLOGY (CRIO), HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. FACULDADE ISRAELITA DE CIÊNCIAS DA SAÚDE ALBERT EINSTEIN, HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL.

Background: High-parameter imaging is key to understanding the spatial complexity of the tumor microenvironment (TME). IBEX (Iterative Bleaching Extends multiplexity) enables highly multiplexed immunofluorescence imaging while preserving tissue architecture, making it ideal for investigating immune-tumor interactions. **Objective:** To implement and optimize the IBEX protocol for identifying immune components and their spatial organization within the TME of human tumor samples from CRIO's prospective and retrospective cohorts. **Methods:** Human tissue sections underwent iterative rounds of antibody staining, confocal imaging, and fluorophore bleaching with lithium borohydride. Acquisitions were performed on the Zeiss LSM 710 confocal microscope. Fixed frozen tissue integrity was preserved using chrome gelatin alum. Image analysis included tissue classification, cell phenotyping, infiltration mapping, and spatial analyses using HALO software. This study was approved by the Research Ethics Committee of the Hospital Israelita Albert Einstein (CAAE: 54281321.6.0000.0071; Approval Number: 6.821.782). **Results:** IBEX was successfully adapted to fixed-frozen and FFPE tissues from CRIO's cohorts, generating high-quality images over multiple cycles. HALO analysis revealed a distinct distribution of CD8⁺ T cells between the tumor and stroma, as well as spatial patterns that distinguished responders from non-responders. The protocol was validated in tonsil and melanoma tissues and applied to cervical, ovarian, and colon cancer samples. **Conclusion:** IBEX is a robust, scalable tool for high-dimensional spatial profiling in human tumors. It enables multiplexed analysis without compromising tissue quality, supporting deep TME exploration. Future steps include standardizing cohort-specific markers, refining image analysis pipelines, and evaluating PhenoCycler-Fusion integration. Supported by FAPESP/GSK (grant number #21/00408-6).

Keywords: Spatial Biology; Multiplex immunofluorescence; Immuno-Oncology.

3301 - Category: TUMOR IMMUNOLOGY (TU)

Local administration of pirfenidone has pro-tumoral effects in an orthotopic model of oral squamous cell carcinoma (OSCC)

FERREIRA, T.D.S.¹; FERNANDES, N.A.R.²; PELEGRIN, Á.F.¹; PASCHOAL, D.¹; ALEMISEREJ, F.¹; REIS, I.B.¹; DE CARVALHO, M.M.¹; NASCIMENTO, C.R.¹; ROSSA JR, C.¹. 1. SÃO PAULO STATE UNIVERSITY (UNESP), ARARAQUARA - SP - BRASIL; 2. UNIVERSITY OF HELSINKI, HELSINKI - FINLANDIA.

Pirfenidone is an antifibrotic drug approved by the FDA for the treatment of idiopathic pulmonary fibrosis (IPF) that inhibits production and activity of TGF- β by activated fibroblasts. Activated fibroblasts of IPF are similar to cancer-associated fibroblasts (CAFs), therefore pirfenidone has also been investigated for cancer treatment. Surprisingly, preliminary data in a zebrafish model showed that systemic administration of pirfenidone, alone or combined with cisplatin, increased metastatic dissemination of oral squamous cell carcinoma (OSCC) cells. In this study, we assessed the effect of intra-tumoral pirfenidone, alone or combined with systemic cisplatin, on tumor progression in a syngeneic immunocompetent orthotopic OSCC mouse model. Thirty C57BL/6 mice were distributed into four groups: Pirfenidone + Cisplatin (Pirf+Cp), Pirfenidone alone (Pirf), Cisplatin alone (Cp), and control ($\emptyset$). On day 0, 1×10^6 MOC2 cells were injected into the floor of the mouth. Mice were weighed every three days and received daily subcutaneous hydration from day 5. On days 7 and 9, pirfenidone (20 μ g in 50 μ L) was administered intra-tumorally and cisplatin (5 mg/kg) intraperitoneally. On day 11, animals were weighted, euthanized and experimental tumors dissected. Total RNA was extracted and expression of IL-6 and TGF- β were evaluated by RT-qPCR. Animals treated with cisplatin showed significant weight loss and reduced tumor volume ($p = 0.0135$) and mass ($p = 0.0258$) compared to $\emptyset$. Pirf was associated with the largest tumor volume and mass. Pirf + Cp also reduced tumor volume and mass compared to $\emptyset$, but less than cisplatin alone. RT-qPCR results showed no significant differences, but a progressive reduction in IL-6 expression was observed in the Cp, P, and P+Cp groups, with a trend of reduced TGF- β expression in the P+Cp group. These findings suggest a pro-tumoral effect of locally administered pirfenidone, alone or combined with systemic cisplatin.

Keywords: pirfenidone; CAFs; OSCC.

3313 - Category: TUMOR IMMUNOLOGY (TU)

THE ROLE OF NLRP3 INFLAMMASOME-DEPENDENT PYROPTOSIS IN THE MODULATION OF CARCINOGENIC AND MITOCHONDRIAL PARAMETERS IN HUMAN GASTRIC CANCER CELLS

MANCHINE, J.P.¹; VIEIRA, M.H.F.¹; MACHADO, S.A.¹; BEZERRA, S.P.¹; DE MELO, H.A.B.¹; ZAMBONI, D.S.²; MAGALHÃES, K.G.¹. 1. UNIVERSITY OF BRASILIA (UNB), BRASÍLIA - DF - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Introduction: Gastric cancer is the fifth most lethal type of tumor worldwide, with no effective treatments available at advanced stages of the disease. This highlights the need to investigate new therapeutic strategies, particularly those targeting cell death pathways, which are often inhibited in cancer as an evasion mechanism. Pyroptosis, a form of lytic programmed cell death, has gained significant attention in oncology due to its dual role in cancer progression depending on the tumor type. However, in gastric cancer, its role remains poorly understood. In this context, this study aims to elucidate the role of NLRP3 inflammasome activation-dependent pyroptosis in modulating carcinogenic parameters in human gastric cancer cells, as well as to assess mitochondrial damage as a secondary mechanism. **Methods:** AGS cells were stimulated with lipopolysaccharide (LPS) (1 µg/mL) for 24 hours and nigericin (20 µM) for 2 hours, followed by either a medium replacement (short stimulation – SS) or continuous stimulation (long stimulation – LS) throughout the analysis periods. Parameters were assessed by spectrophotometry, flow cytometry, and fluorescence microscopy. **Results:** Both SS and LS induced membrane pore formation, which is associated with cathepsin B release and reactive species production, and caspase-1 activation, with the effect being intensified in LS, which also showed increased lactate dehydrogenase (LDH) release. Additionally, both stimuli reduced the number of cells in the G2/M phase, cell viability, and proliferation, while increasing lytic cell death, and the effects were more pronounced in LS. Both SS and LS increased reactive species production and reduced mitochondrial abundance. **Conclusions:** Our findings demonstrate a potential antitumor effect of pyroptosis induction in gastric cancer *in vitro*, unveiling novel perspectives to support the development of new therapeutic approaches against gastric cancer.

Keywords: gastric cancer;pyroptosis;mitochondria.

3314 - Category: TUMOR IMMUNOLOGY (TU)

Immune Profiling and Biomarker Discovery in Retrospective and Prospective Cervical Cancer

NUNES, C.M.D.C.¹; MORAIS, K.L.P.¹; MARIANO, R.M.D.S.¹; APOSTÓLICO, J.¹; CORTES, L.G.F.¹; MESSIAS, I.M.¹; BARONI, J.S.C.¹; IOHAN, L.¹; MARTINELLI, M.¹; MARQUES, R.M.²; BEZERRA, V.A.²; DE SOUZA, A.F.N.²; DE MOURA, V.E.²; BRITTO, A.C.D.C.²; DEFELICIBUS, A.³; DA SILVA, I.T.³; GOLLOB, K.J.¹. 1. INSTITUTO ISRAELITA DE ENSINO E PESQUISA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 3. A.C. CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

Introduction: Cervical cancer remains a major public health concern, particularly in low- and middle-income countries. Most cases are driven by persistent infection with high-risk human papillomavirus (HPV), especially types 16 and 18. While tumor-specific alterations like genomic instability and mutational burden play a role in disease progression, immune responses, both local and systemic, can also influence treatment outcomes.

Objectives: To identify immune-related molecules, pathways, and cellular networks associated with treatment response in cervical cancer, using both prospective and retrospective patient cohorts.

Methods: Patients with FIGO stage IB3–IVA cervical cancer treated with chemotherapy (with or without radiotherapy or brachytherapy) were categorized as responders (R) or non-responders (NR). In the retrospective cohort (n=26), tumor samples underwent whole-exome sequencing (WES), and HPV detection/genotyping was performed using HPV-KITE. In the prospective cohort (n=18), plasma samples collected at multiple timepoints were analyzed for 32 immune mediators using a Milliplex assay.

Results: Preliminary WES findings revealed alterations in genes involved in DNA repair and immune regulation. HPV subtypes were detected in cervical cancer patient samples, and no differences between R and NR were observed in the coverage of HPV16 and HPV18. In the prospective cohort, responders exhibited consistently higher plasma levels of IL-1α and IL-7 at baseline and throughout treatment.

Conclusions: Elevated IL-1α and IL-7 levels in R patients reflect distinct aspects of the systemic immune environment, involving inflammatory responses and T-cell homeostasis. To further explore the interplay between HPV-related factors and immune pathways, we plan to apply complementary approaches in future analyses, such as calculating HPV read counts per million and assessing viral load via PCR.

Keywords: Cervical cancer;Immune response;HPV.

3330 - Category: TUMOR IMMUNOLOGY (TU)

Effects of Extracellular Vesicles Derived from 4T1 Cells on the Viability of RAW264.7 Macrophages.

FLORIANO, G.C.; LIMA, M.J.T.; JANUZZI, L.L.R.; VENERANDO, L.A.F.; SOARES, S.M.A.; GAMEIRO, J..
UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Solid tumors have the ability to progressively remodel the immune microenvironment, favoring their own survival and dissemination. Recent studies have highlighted the role of extracellular vesicles (EVs) as important mediators of tumor progression, metastasis, and host immunosuppression. Derived from cancer cells, EVs can interact with immune cells, modulating their activity. This study evaluated the effects of EVs from the 4T1 breast cancer cell line on the viability of RAW264.7 macrophages. 4T1 cells were cultured in EV-depleted medium. The supernatant was collected and subjected to differential centrifugation, including ultracentrifugation, to isolate the EVs. Particle size was analyzed via dynamic light scattering (Zetasizer), and protein content by BCA assay. Macrophages (5×10^3 cells/well) were treated with 10, 15, and 20 $\mu\text{g/mL}$ of EVs, and cell viability was assessed using the MTT assay at 24 h and 48 h. Vesicle diameters were 203.2 nm and 151.7 nm, corresponding to microvesicles and exosomes, respectively. BCA results showed higher protein levels in microvesicles. After 24 h, microvesicles at 10 $\mu\text{g/mL}$ significantly increased cell viability, whereas exosomes at the same concentration and both types of vesicles at 15 $\mu\text{g/mL}$ did not cause significant changes compared to the control, suggesting no major metabolic alterations. Exosomes at 20 $\mu\text{g/mL}$ showed a trend toward increased mitochondrial activity. At 48 h, microvesicles at 10 $\mu\text{g/mL}$ markedly increased viability, suggesting a proliferative stimulus; exosomes also increased viability at this dose, but to a lesser degree. Higher concentrations showed no major increases, and exosomes at 20 $\mu\text{g/mL}$ significantly reduced mitochondrial activity, suggesting a dose-dependent toxic effect. These findings indicate that 4T1-derived EVs modulate RAW264.7 macrophage viability in a dose and time-dependent manner. Future studies will explore their role in macrophage polarization (M1/M2) and tumor immunomodulation.

Keywords: extracellular vesicles;tumor immunomodulation;Breast Cancer.

3340 - Category: TUMOR IMMUNOLOGY (TU)

Characterization of plasma extracellular vesicles from obese women and their effects on the MDA-MB-231 breast tumor cell line

LIMA, M.J.T.; VENERANDO, L.A.F.; DIB, P.R.B.; JANUZZI, L.L.R.; FERNANDES, M.K.C.; HOTTZ, E.; PERCEGONI, N.; GAMEIRO, J.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Much is said about obesity, a disease characterized by metainflammation sustained by adipose tissue. Its interrelation with other pathologies is explored, and breast cancer emerges as one of the diseases associated with obesity. This condition plays an important role in the development of the tumor microenvironment, explored through extracellular vesicles (EVs), which are nanometric particles secreted by cells and found in body fluids. The main objective of this project is to clarify the effect of circulating EVs on the malignancy of breast tumor cells through isolation, characterization, and study of cell viability, migration, and expression of pro-tumor genes in MDA-MB-231 cells. EVs were isolated from the plasma of obese women using Size Exclusion Chromatography (SEC), and their size was measured by Dynamic Light Scattering (DLS). Protein concentration was measured by the BCA assay, and cholesterol levels were also determined. Samples were obtained from obese patients at UFJF University Hospital and from eutrophic volunteers. Tumor cells were exposed to EVs in MTT and colony-formation assays. We observed no correlation between protein concentration and degree of obesity. Results suggest that EVs from obese women are larger (>100 nm) than those from normal-weight women (<100 nm). Lower mitochondrial activity ($p = 0.0271$) was observed in tumor cells treated with EVs from obese women ($n = 10$; 150%) compared with cells treated with eutrophic EVs ($n = 7$; 225%), supporting a shift toward the lactate pathway. Cells treated with EVs from obese women showed greater colony-forming capacity ($n = 5$; 38 colonies) than cells treated with EVs from eutrophic women ($n = 2$; 27 colonies), although this finding needs confirmation. Therefore, further analysis needs to be done, but now these results suggest that circulating EVs from obese patients tend to promote tumor development and increase tumor cell malignancy, highlighting the relevance of extracellular vesicles in obesity.

Keywords: Obesity;Cancer;Extracellular vesicles.

3364 - Category: TUMOR IMMUNOLOGY (TU)

Systemic Immune Signatures and Tumor Mutational Profiles Associated with Therapy Response in Ovarian Cancer

MARIANO, R.M.D.S.¹; CORTES, L.G.F.¹; MESSIAS, I.M.¹; BARONI, J.S.C.¹; NUNES, C.M.D.C.¹; IOHAN, L.¹; MORAIS, K.L.P.¹; APOSTÓLICO, J.¹; MARTINELLI, M.¹; MARQUES, R.M.²; BEZERRA, V.A.²; DE SOUZA, A.F.N.²; DE MOURA, V.E.²; BRITTO, A.C.D.C.²; DEFELICIBUS, A.³; DA SILVA, I.T.³; GOLLOB, K.J.¹. 1. ALBERT EINSTEIN INSTITUTO ISRAELITA DE ENSINO E PESQUISA, SÃO PAULO - SP - BRASIL; 2. HOSPITAL ISRAELITAALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 3. A.C.CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

Background: Ovarian cancer is the most lethal gynecological malignancy. For patients diagnosed at advanced stages, the 5-year survival rate is ~41%, and fewer than 15% survive beyond 10 years. Despite recent advances, not all patients benefit from targeted therapies, and effective treatment options remain limited. **Objectives:** To identify immune-related molecules, pathways, cell types, and networks associated with treatment response in ovarian cancer. **Methods:** Patients with high-grade stage III–IV ovarian carcinoma were included. The classification as responders or non-responders to treatment was performed only in the retrospective cohort. In the prospective cohort (n=6), plasma samples were analyzed using a multiplex bead array to measure ~30 immune-related analytes. In the retrospective cohort (n=24), tumor samples underwent whole-exome sequencing (WES) to assess mutational burden and identify differentially expressed genes between groups. **Results:** Ovarian patients have higher levels of pro-inflammatory cytokines (IL-1 β , IL-12p40, IL-12p70, TNF- α) and chemokines (IL-8, IP-10, MIP-1 β , MCP-3 and RANTES), as well as anti-inflammatory and immunoregulatory cytokines (IL-4, IL10 e IL-1RA) and growth factors (FGF-2 and G-CSF) when compared to healthy controls. This dual profile reflects a complex systemic immune response. Preliminary WES findings revealed alterations in genes involved in DNA repair and immune regulation. **Conclusion:** The presence of pro- and anti-inflammatory cytokines in patient plasma suggests potentially regulatory attempt by the systemic immune response in ovarian cancer. Circulating growth factors may also modulate the tumor microenvironment by influencing immune activity and angiogenesis. Integration of WES data with clinical outcomes is ongoing to identify biomarkers predictive of treatment response and identify potential targets for improving immune responses against ovarian cancer.

Keywords: ovarian cancer;immune response;biomarkers.

3388 - Category: TUMOR IMMUNOLOGY (TU)

The anti-proliferative additive effect of combining BCL-2 and GSK3 β inhibitors in acute myeloid leukemia

HUMMEL, G.A.¹; KIMMEMGS, T.N.²; PANSONATO, .L.¹; NETO, J.D.A.¹; AMANO, M.T.¹. 1. INSTITUTO DE ENSINO E PESQUISA HOSPITAL SÍRIO LIBANÊS (IEP), SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Acute myeloid leukemia (AML) remains a significant challenge with low cure rates. The anti-apoptotic BCL-2 protein, frequently overexpressed in hematological malignancies, promotes leukemic cell survival. Although BCL-2 inhibitors, such as Venetoclax (VEN), are already approved, the primary or acquired resistance limits their efficacy. Given the role of GSK3 β in AML progression and the promising in vitro results of its inhibitors, our aim is to investigate the effect of the combination of VEN and BIO (a GSK3 β inhibitor) to improve anti-leukemic effect in AML cell lines. We used HL-60, HEL, MOLM-13, and U937 AML cell lines for in vitro studies, performing proliferation, cell death, and gene expression assays. In IC50 assays, HEL and U937 cell lines were less sensitive to VEN than HL-60 and MOLM-13. However, the combined treatment consistently reduced the cell population in all AML cell lines, suggesting an additive effect. No changes were observed in pro-apoptotic genes, whereas the combined treatment altered GSK3 β expression in HL-60 and MOLM-13 cell lines, while in the HEL cell line, it altered BCL-2 expression. Although HEL cells are less sensitive to VEN, death assays showed that VEN alone induced a significant cell death. However, we did not observe an additive effect between BIO and VEN in the combined treatment. In contrast, an additive effect was observed in MOLM-13, HL-60 and U937 cells when treated with the combination. Our results demonstrate that the combined treatment effectively reduces cell proliferation and enhances cell death in different AML cell lines, including those less sensitive to VEN. Importantly, this efficacy is achieved through distinct and cell line-specific molecular mechanisms, indicating a broad modulation of different pathways, highlighting possibilities for therapeutic application in the future.

Keywords: Acute myeloid leukemia (AML);BCL-2;GSK3b.

3392 - Category: TUMOR IMMUNOLOGY (TU)

CHEMOTHERAPY SEQUENCE MODULATES IMMUNE LANDSCAPE: NK CELL ACTIVATION IN NEOSAMBA

SANTOS, I.D.O.¹; DOURADO, L.P.S.²; DUARTE, M.S.V.³; ARAUJO, E.V.D.O.⁴; KINKER, G.S.⁴; ARANTES, L.M.R.B.⁵; RODRIGUES, F.R.²; MEDINA, T.⁵; BONAMINO, M.H.⁶. 1. UNB/INCA, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL; 3. CELLURIS, SÃO PAULO - SP - BRASIL; 4. A.C. CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 5. A. C. CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 6. INSTITUTO NACIONAL DE CÂNCER (INCA); FUNDAÇÃO OSWALDO CRUZ(FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

Breast cancer chemotherapy typically involves the sequential use of anthracyclines (FAC) followed by taxanes (T). However, the NeoSAMBA clinical trial demonstrated that the reverse sequence, T followed by FAC (T-FAC), led to improved disease-free and overall survival. This study focused on the impact of neoadjuvant chemotherapy T followed by FAC on leukocyte infiltration and gene expression profiles in breast tumors of patients enrolled in the trial. We analyzed clinical samples from the NeoSAMBA trial to investigate the impact of the T-FAC chemotherapy sequence on the tumor immune landscape. Using NanoString technology, we assessed the modulation of immune-related gene expression. We also performed immunohistochemistry to evaluate the infiltration of NK (CD56) and B cells (CD20), as well as the formation of tertiary lymphoid structures (TLS). Our analysis revealed distinct immune modulations driven by the chemotherapy sequence. The T-FAC sequence significantly increased the expression of genes associated with NK cell activation, while the standard FAC-T sequence upregulated genes related to NK cell inhibition. This was corroborated by immunohistochemistry, which showed a higher number of NK cells in T-FAC-treated tumors. Furthermore, the T-FAC sequence enhanced the expression of genes involved in innate and adaptive immunity, while downregulating genes related to basic cellular functions and chemokines. No significant difference was observed in B cell numbers between the groups. The T-FAC sequence promotes a more robust and favorable immune landscape, primarily through the activation of NK cells. These findings offer a potential biological explanation for the improved patient outcomes observed in the NeoSAMBA trial and highlight the importance of chemotherapy sequence in modulating anti-tumor immunity. Further studies are underway to fully elucidate the clinical implications of these immune modulations.

Keywords: Breast Cancer;Neoadjuvant Chemotherapy;NeoSAMBA Trial.

3395 - Category: TUMOR IMMUNOLOGY (TU)

MAPPING THE IMMUNE LANDSCAPE IN PEDIATRIC B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: PROGNOSTIC SIGNALS FOR MORTALITY

MAGALHÃES-GAMA, F.¹; DA SILVA, F.S.²; MORAES, J.D.S.²; NEVES, J.C.F.³; ROCHA, I.A.D.C.⁴; FERNANDES, G.D.R.¹; CARVALHO, M.D.P.S.S.⁵; TARRAGÔ, A.M.⁵; MALHEIRO, A.²; MARTINS-FILHO, O.A.⁴; DA COSTA, A.G.²; CARVALHO, A.T.⁴. 1. PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE (PPGCS), INSTITUTO RENÉ RACHOU - FUNDAÇÃO OSWALDO CRUZ (IRR-FIOCRUZ), BELO HORIZONTE - MG - BRASIL; 2. PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA BÁSICA E APLICADA (PPGIBA), UNIVERSIDADE FEDERAL DO AMAZONAS (UFAM), MANAUS - AM - BRASIL; 3. PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA TROPICAL (PPGMT), UNIVERSIDADE DO ESTADO DO AMAZONAS (UEA), MANAUS - AM - BRASIL; 4. GRUPO INTEGRADO DE PESQUISAS EM BIOMARCADORES (GIPB), INSTITUTO RENÉ RACHOU - FUNDAÇÃO OSWALDO CRUZ (IRR-FIOCRUZ), BELO HORIZONTE - MG - BRASIL; 5. DIRETORIA DE ENSINO E PESQUISA (DEP), FUNDAÇÃO HOSPITALAR DE HEMATOLOGIA E HEMOTERAPIA DO AMAZONAS (HEMOAM), MANAUS - AM - BRASIL.

Background: B-cell Acute Lymphoblastic Leukemia (B-ALL) is the most common pediatric cancer, but challenges persist in predicting outcomes for a subset of patients who experience relapse or treatment-related mortality. The role of the systemic immune response in these adverse outcomes remains unclear. Therefore, this study aimed to characterize the dynamic immune landscape and its prognostic value in pediatric B-ALL. **Methods:** For this, a comprehensive profiling of 48 soluble mediators, including chemokines, pro-inflammatory cytokines, and growth factors, was performed in the peripheral blood (PB) and bone marrow (BM) of 36 pediatric B-ALL patients and 25 healthy controls. Using multiplex assays, we conducted a longitudinal analysis with samples collected at diagnosis (D0) and at three key time points during remission-induction chemotherapy (D15, D35, and D84). **Results:** Our findings revealed a broadly exacerbated systemic immune profile at diagnosis (D0), followed by rhythmic variations and a delayed normalization of most mediators during treatment. We identified a persistent dysregulation of the immune interaction network. Noteworthy, the exacerbated immune profile at D0 strongly preceded mortality, and this was validated by the identification of specific mediator panels with high predictive accuracy for this outcome: the panel from PB included CCL5, IL-6, CXCL9, and IL-2R α , while the panel from BM included CXCL12 and CCL4. These panels predicted mortality with AUC values of 0.996 in PB and 0.900 in BM, respectively. **Conclusion:** The dynamic and dysregulated immune landscape identified in this study provides a crucial new framework for understanding pediatric B-ALL. By offering novel prognostic signals, these findings not only shed light on host-tumor interaction but also lay the groundwork for developing tailored immunomodulatory strategies and enabling truly personalized risk stratification.

Keywords: pediatric leukemia;immunodysregulation;biomarkers.

3426 - Category: TUMOR IMMUNOLOGY (TU)

Evaluation of SARS-CoV-2 Infection on Colorectal Cancer Progression in 2D and 3D Models

MOREIRA, I.B.G.¹; SOARES, V.C.¹; WERNECK, M.B.F.¹; MASCARENHAS, G.D.¹; DE CAMPOS, M.M.¹; DE OLIVEIRA, D.M.¹; LIMA, G.T.D.C.F.²; ALVES, M.C.S.²; SANTAREM, G.N.²; DIAS, S.D.S.G.²; SOUZA, T.M.L.²; BOZZA, P.T.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Colorectal cancer (CRC) is a significant global health concern, ranking third in incidence and second in cancer-related mortality. Its pathogenesis involves complex interactions among genetic mutations, chronic inflammation, and metabolic reprogramming, collectively promoting tumor progression via angiogenesis, invasion, and metastasis. The tumor microenvironment, shaped by sustained inflammatory signaling and altered lipid metabolism, plays a critical role in CRC development. During the COVID-19 pandemic, systemic effects of SARS-CoV-2 have been linked to disruptions beyond the respiratory system, with emerging evidence suggesting modulation of tumor biology. However, the impact of SARS-CoV-2 on CRC remains unclear. This study investigates SARS-CoV-2 infection effects on CRC using 2D monolayer and 3D spheroid co-culture models of HCT116 colorectal carcinoma and BJ5Ta fibroblast cell lines. In 2D cultures, infection at MOIs 0.01, 0.1, and 1 did not induce cytopathic effects or cell death despite ACE2 and TMPRSS2 expression, essential viral entry factors. Detection of double-stranded RNA and lipid droplet accumulation in both cell lines suggests active viral replication and metabolic remodeling. In 3D co-cultures, infection at MOI 0.1 was performed two days post-spheroid formation. At 2 days post-infection, IL-6 secretion increased in infected spheroids, indicating an acute inflammatory response, while VEGF levels remained unchanged. By day eight, IL-6 decreased and VEGF increased similarly in infected and control spheroids, consistent with hypoxia core formation. Despite these cytokine changes, there were no differences in spheroid growth kinetics between the groups. These findings suggest SARS-CoV-2 may modulate the CRC microenvironment through inflammatory and metabolic pathways on tumor growth in vitro. The cytokine dynamics observed imply potential roles in tumor progression, regarding further investigation into SARS-CoV-2's influence on CRC pathophysiology.

Keywords: SARS-CoV-2; Cancer progression; Inflammation.

3443 - Category: TUMOR IMMUNOLOGY (TU)

ANTI-HER2 CAR-T CELLS EVALUATION IN AN OVARIAN TUMOR PRECLINICAL MODEL

ARAGAO, E.A.A.; ABDO, L.D.M.; VIEIRA, L.M.; CORREIA, E.M.; BONAMINO, M.H.. INCA, RIO DE JANEIRO - RJ - BRASIL.

In CAR-T cell therapy, T lymphocytes are genetically modified to express a chimeric antigen receptor (CAR), enabling tumor recognition and activation of an antitumor immune response. This approach has transformed hematological cancer treatment, offering options for patients refractory to conventional therapies. However, its application in solid tumors remains limited, requiring further research. A major challenge in anti-HER2 CAR-T therapy—targeting an antigen present in various tumors—is selecting the optimal extracellular domain for antigen recognition. This study aimed to evaluate and compare the efficacy of producing two anti-HER2 CARs (4D5 and FRP5) in peripheral blood mononuclear cells (PBMCs), as well as their antitumor capacity in vitro and in vivo. PBMCs were electroporated using a 2:1 transposase-to-transposon ratio and cultured for 12 days. The resulting CAR-T cells were analyzed by flow cytometry and tested functionally. CAR expression (~25%) and a predominant central memory phenotype (CD45RO⁺ CCR7⁺) were observed, with low exhaustion, especially in CD8⁺ FRP5⁺ cells. CAR-4D5 showed greater antitumor activity, inducing more SK-OV-3 and U2OS cell lysis and higher IL-2, TNF- α , and IFN- γ production. In vivo, NSG mice with SK-OV-3 tumors received 8.9×10^6 cells (1.3×10^6 CAR⁺). Tumor regression occurred in the CAR-4D5 group, but necrosis led to fatalities; no response was seen with CAR-FRP5. A second experiment tested different administration routes in early-stage tumors. Intraperitoneal injection showed the best outcome, with 4/5 mice tumor-free after one year and no toxicity, while other routes caused side effects and reduced survival. Both CARs showed central memory phenotypes and low inhibitory receptor expression. CAR-4D5 was more effective, especially via intraperitoneal delivery.

Keywords: Immunotherapy; CAR-T; HER2.

3446 - Category: TUMOR IMMUNOLOGY (TU)

Evaluation and Characterization of Tumor-Infiltrating Lymphocytes (TILs) Isolated from Patients with Acral Melanoma

DO NASCIMENTO, R.M.C.¹; SILVA, L.R.B.²; DE OLIVEIRA, J.L.¹; BONAMINO, M.H.¹; POSSIK, P.A.¹. 1. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

Acral melanoma (AM) is a rare, aggressive melanoma subtype that affects glabrous skin (palms, soles, subungual regions). Its frequency among subtypes is higher in Latin Americans than in populations of European descent. Often diagnosed at advanced stages, AM is associated with poor prognosis and limited treatment options. Adoptive cell therapy (ACT) using ex vivo expanded tumor-infiltrating lymphocytes (TILs) was recently approved by the FDA, demonstrating efficacy in patients with advanced melanoma, including those refractory to immune checkpoint inhibitors (ICIs). However, its application in AM is underexplored due to scarce functional characterization of TIL and limited clinical data of AM patients' response to ACT. This study aims to isolate, expand, and characterize TILs from AM patients treated at the INCA and to evaluate their therapeutic potential. Tumor samples from six patients were processed either by enzymatic dissociation or direct fragment culture with high-dose interleukin-2 (6,000 IU/mL). TIL cultures were monitored for morphology and expansion, and analyzed by flow cytometry. In parallel, tumor fragments were implanted into immunocompromised mice to generate autologous patient-derived xenografts (PDX) and cell lines. TILs were successfully expanded from all samples. Those from four patients were characterized, showing a predominance of CD45+CD3+ T cells (mean: 84.6%, SD: 17.1). CD4+ and CD8+ cells averaged 46.1% (SD: 27.6) and 47.3% (SD: 27.1), respectively. Regulatory T cells were rare (mean: 0.6%, SD: 1.6). PD-1 expression was high in three samples (mean: 93.2%, SD: 32.6), while LAG-3 and TIM-3 were expressed at lower levels (LAG-3 mean: 0.3%, SD: 2.7; TIM-3 mean: 6.5%, SD: 5.2). PDX models were established from three patients, and PDX-derived cells are being expanded for co-culture assays. These results confirm the feasibility of TIL expansion and generation of PDXs and cell cultures from AM samples, providing a platform for pre-clinical studies.

Keywords: acral melanoma; tumor-infiltrating lymphocytes (TILs); adoptive cell therapy (ACT).

3457 - Category: TUMOR IMMUNOLOGY (TU)

EZH2 marks a CD8 T Cell Dysfunction and Immunotherapy Resistance in cancer

DE OLIVEIRA, J.P.D.L.; PASSOS, T.D.O.; PEREIRA, R.D.M.S.. IMMUNOLOGY AND INFLAMMATION DEPT., IMPG, UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Alternative differentiation trajectories of dysfunctional CD8 T cells have been described within tumors and, like the classical memory/effector model, rely on chromatin remodeling. *EZH2*, a histone methyltransferase involved in T cell fate, remains poorly characterized in this context. Here, we identify *EZH2* as a marker of proliferation in dysfunctional CD8 T cells and a negative prognostic factor in cancer immunotherapy.

We analyzed scRNA-seq data from melanoma tumors before and after anti-PD-1 therapy to define the states of CD8 T cells. Seurat was used for clustering and normalization. Gene signature, module-level, and pseudotime analyses (by Monocle) revealed proliferative dysfunctional subsets and intratumoral dynamics. Conservation was assessed using LCMV CI13RNA-seq data, a well-established system for T cell exhaustion.

In melanoma, *EZH2* expression was enriched in highly proliferative, exhausted CD8 T cell clusters, suggesting the presence of a distinct trajectory toward terminal dysfunction. This *EZH2*-enriched cluster not only exhibited high levels of proliferation-associated genes but also showed moderate expression of canonical exhaustion markers, including *TOX*, *TOX2*, *PDCD1*, and *CTLA4*. Conversely, it was characterized by low expression of memory and stem-like genes such as *TCF7*, *SELL*, *BCL2*, and *IL7R*. *EZH2* expression was significantly higher in non-responders compared to responders within the CD8 T cell compartment. These observations were mirrored in the LCMV clone 13 chronic infection model, where *Ezh2* expression correlated negatively with progenitor-associated genes and positively with proliferation.

We identified *EZH2* as a marker of proliferative, dysfunctional CD8 T cells linked to resistance to anti-PD-1 immunotherapy. This suggests a role for *EZH2* in driving the dysfunctional phenotype. These findings support the rationale for targeting *EZH2* as a strategy to modulate CD8 T cell states and enhance responses to immunotherapy.

Keywords: Tumor; Lymphocyte; Epigenetics.

3458 - Category: TUMOR IMMUNOLOGY (TU)

Study of the cytotoxicity of *Plinia cauliflora* extract in breast cancer cell line

ANDRADE, J.D.S.; DE PAOLI, F.; GAMEIRO, J.; FABRI, R.L.; LIMA, M.J.T.; ROCHA, V.N.. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL.

Breast cancer is the leading cause of cancer-related mortality among women in Brazil. In the search for therapeutic alternatives, several natural compounds have been investigated for their antitumor potential. *Plinia cauliflora*, popularly known as jabuticaba, is a species native to the Brazilian Atlantic Forest, rich in phenolic compounds with antioxidant, anti-inflammatory, antimicrobial and antitumor properties. The aim of the present study was to evaluate the cytotoxicity of the extract obtained from the branch of *P. cauliflora* in the human breast carcinoma cell line MDA-MB-231 *in vitro*. Cells (5×10^4 per well) were treated with different concentrations of the extract (300, 150, 75, 37.5, 18.7 and 9.3 $\mu\text{g/mL}$) for 24 hours, and cytotoxicity was assessed using the MTT assay. A progressive decrease in cell viability was observed with increasing extract concentration, indicating a dose-dependent response. Treatment with the highest concentration (300 $\mu\text{g/mL}$) resulted in a 70% reduction in cell viability compared to the control group. These findings suggest that the branch extract of *P. cauliflora* possesses therapeutic potential and may serve as a promising candidate for expanding breast cancer treatment options. In addition to being rich in bioactive compounds with selective cytotoxicity that does not affect healthy cells, it is found abundantly throughout Brazil. Future studies will investigate its effects on cell migration to further assess its impact on tumor progression in triple-negative breast cancer cell line.

Keywords: Breast cancer; Triple-negative breast cancer cell line; *Plinia cauliflora*.

3462 - Category: TUMOR IMMUNOLOGY (TU)

Generation of CAR-NK92mi cells as an off-the-shelf approach using nonviral minimized vectors

ALVES, P.D.S.; ARAGAO, E.A.A.; BONAMINO, M.H.. INSTITUTO NACIONAL DE CANCER, RIO DE JANEIRO - RJ - BRASIL.

The autologous CAR-T cell therapy is highly effective against certain hematologic tumors but raises concerns regarding safety and cost. CAR-NK92 cells, tested in clinical trials for CAR-based therapies, offer allogeneic use and a lower risk of adverse effects. The NK-92mi clone is an IL-2-independent NK cell line that reduces costs to expand and modify, make it a practical off-the-shelf CAR carrier. In consonance, to reduce costs related to viral vector scalability, in this present work were tested two transposon/transposase-based systems: Sleeping Beauty (SB) and piggyBac (PB) to generate anti-CD19 CAR-NK92mi cells. In the SB system, were used PT3, PT4, and an innovative minimized version of PT4 vector called minicircle (PT4-MC). These transposons were combined with the SB100x transposase, as well as the PB was used with the PBase transposase. For anti-CD19 CAR+ cell generation, 1×10^6 of NK92mi cells were separated for each condition (mock, PT3, PT4, PT4-MC, and PB) and electroporated with the 2b Nucleofector (Lonza) using the NK92 program. Subsequently, viability and the presence of anti-CD19 CAR were assessed by flow cytometry. As expected, NK92mi cells were highly sensitive to electroporation; the viability in all conditions was reduced to approximately 20% of the initial cell count. Moreover, while the other plasmids did not demonstrate transfection efficiency, the PT4-MC frequency of 5% CAR-NK92mi was stable. Thus, the transfection methodology with minimized vectors demonstrates better transfection efficiency and stability of transgene integration. NK cells own innate immunity mechanisms that impair the transfection process. In this way, our results suggest the reduced size of the minicircle nonviral vectors may have advantage in the genetic modification of NK cells. Enrichment assays for CAR-NK92mi cells are underway, as well as *in vitro* and *in vivo* cytotoxic activity against B-cell cancer cell lines and xenograft mouse model, respectively.

Keywords: Células natural killer; CAR-NK; Imunoterapia.

3472 - Category: TUMOR IMMUNOLOGY (TU)

DEVELOPMENT AND IN VITRO CHARACTERIZATION OF A 4TH GENERATION CAR-T CELL PRODUCT FOR ACUTE MYELOID LEUKEMIA

DE SOUZA, I.F.; SUZUKI, C.I.; VIDAL, E.K.D.S.; MITSUGI, T.G.; FERREIRA, R.A.; SILVA, L.A.M.T.; DA SILVA, J.R.; BARROS, C.D.C.O.; HAMERSCHLAK, N.; ZANETTI, L.C.; KERBAUY, L.N.. FACULDADE ISRAELITA EM CIÊNCIAS DA SAÚDE ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL.

Acute Myeloid Leukemia (AML) is an aggressive hematological malignancy marked by limited treatment options and poor prognosis, particularly in relapsed or refractory patients. Despite advances in Chimeric Antigen Receptor (CAR)-T cell therapy for lymphoid neoplasms and multiple myeloma, its application to AML remains challenging due to the lack of tumor-specific antigens and the immunosuppressive microenvironment. In this context, AML-15 has emerged as a promising target, given its high expression in leukemic blasts and minimal expression in normal hematopoietic cells. For confidentiality and intellectual property reasons, the molecular identity of AML-15 will not be disclosed in this study. In this study, we developed and evaluated a fourth-generation CAR-T cell construct, incorporating Interleukin-15 (IL-15) cytokine, to improve efficacy and persistence. Healthy donors T cells were isolated, activated and transduced with lentivirus encoding the anti-AML-15 CAR. These CAR-T cells were subsequently expanded and analyzed by flow cytometry. Functional assays then assessed their specific cytotoxicity against AML-15 positive or negative tumor cell lines. Primary human T cells were efficiently transduced with the anti-AML-15 CAR, achieving high transduction rates ($93.98\% \pm 0.65$, $n=5$) and robust expansion (100–350 fold) with $>90\%$ viability over 14 days. In functional assays, CAR-T cells showed specific cytotoxicity against AML-15+ cell lines (THP-1, $n=4$; MOLM-13, $n=1$), reaching $\sim 60\%$ lysis at a 10:1 E:T ratio, while untransduced (UT) T cells showed minimal effect. CAR-T cells co-cultured with THP-1 produced significantly higher TNF α and IFN γ compared to UT T cells ($p<0.05$). We successfully developed a fourth-generation anti-AML-15 CAR-T with potent and specific in vitro cytotoxicity and cytokine production, representing an important step toward accessible immunotherapy for AML in Brazil.

Keywords: Cell Therapy;Chimeric Antigen Receptor;Acute Myeloid Leukemia.

3488 - Category: TUMOR IMMUNOLOGY (TU)

Genomic and Immunologic Profiling of Non-Small Cell Lung Cancer: Identifying Biomarkers of Immunotherapy Response in a Southern Brazil Cohort

MARQUES, C.G.D.S.¹; CORRÊA, B.¹; DA SILVA, A.B.¹; NUNES, C.P.¹; DE MATTOS, G.F.P.¹; FROZZA, F.T.B.¹; GASSEN, R.B.¹; SCHUCK, A.B.¹; SODER, S.²; BONORINO, C.¹. 1. UFCSPA, PORTO ALEGRE - RS - BRASIL; 2. SANTA CASA DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL.

Heterogeneous response to immunotherapy in non-small cell lung cancer (NSCLC) represents a central challenge. Understanding how specific driver mutations shape the tumor immune microenvironment is critical for advancing patient care. This study aims to integrate genomic and transcriptomic profiles from a Southern Brazil NSCLC cohort to define molecular signatures that link driver mutations to the tumor immune microenvironment, thereby identifying biomarkers for immunotherapy stratification. Tumor DNA and RNA from patient samples underwent Next-Generation Sequencing. For high-confidence somatic variant detection from tumor-only data, we applied a robust GATK4 pipeline. The workflow includes BAM pre-processing with base quality score recalibration (BQSR), variant calling via MuTect2, and a stringent dual-filtering strategy. High-confidence variants were comprehensively annotated, and key findings are being verified using Thermo Fisher's Ion Reporter platform. This initial high-confidence analysis of 13 patients identified pathogenic variants in key driver genes, including KRAS (39%), EGFR (23%), and MET (8%), with 62% of patients harboring at least one targetable mutation. Regarding immune response biomarkers, no patient presented high TMB, except one intermediate case (11.42 mut/Mb), and all patients presented microsatellite stability. As a next step, a multi-omic integration of these genomic findings with bulk RNA-sequencing profiles and clinical data will allow us to stratify the cohort and identify significant associations with the tumor immune microenvironment, such as changes in immune cell infiltration and cancer pathway activity. Furthermore, ongoing flow cytometry analyses will provide a deeper characterization of immune cell subsets, enabling a direct correlation between specific driver mutations and patient immunophenotypes.

Keywords: NSCLC;Driver Mutations;Tumor Microenvironment.

3503 - Category: TUMOR IMMUNOLOGY (TU)

IMMUNOMODULATORY AND ANTITUMOR EFFECTS OF SNAKE VENOMS IN GLIOBLASTOMA CELLS: A PRELIMINARY RESEARCH

TUMBACO, C.E.R.¹; GIRARDI, C.S.¹; VIEIRA, O.J.¹; BRAUN, R.L.¹; DE ALMEIDA, J.R.²; BRAGANHOL, E.¹. 1. UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL; 2. UNIVERSITY OF READING, READING - INGLATERRA.

Glioblastoma is the most aggressive primary brain tumor, characterized by high proliferation, invasiveness, and resistance to conventional therapies. While cytotoxicity remains a key parameter, emerging evidence emphasizes the importance of tumor immune interactions in therapy resistance and progression. Snake venoms are rich in bioactive peptides and proteins with cytotoxic, pro-apoptotic, antiangiogenic, and potentially immunomodulatory properties, making them promising candidates for antitumor and immunotherapeutic applications. This study aimed to evaluate the antitumor and possible immunomodulatory effects of ten snake venoms on glioblastoma cells, as a basis for identifying peptide based compounds for combined oncological and immune targeted strategies. Lyophilized venoms from *Agkistrodon piscivorus*, *B. asper*, *B. atrox*, *Bothrocophias microphthalmus*, *C. cerberus*, *C. mitchellii pyrrhus*, *C. o. oreganus*, *C. o. helleri*, *C. cerastes*, and *L. muta* were tested in U138MG glioblastoma cells. Treatments were performed with concentrations from 0.3 to 100 µg/mL for 48 h. Cell viability was evaluated by MTT assay, and IC₅₀ values determined. *Agkistrodom p.* and *B. asper* venom showed the highest cytotoxicity (IC₅₀: 5.4 and 7.5 µg/mL). Phalloidin staining demonstrated marked cytoskeletal disruption, suggesting actin disorganization as a mechanism of cell death. All venoms were analyzed by RP-HPLC, revealing complex peptide and protein profiles. Based on cytotoxicity, selected venoms are under biochemical and proteomic analysis. Conditioned media from treated cultures were collected for ELISA and flow cytometry to assess secretion of key cytokines (IL-6, TNF-α, IL-10, TGF-β). This approach will determine if venom exposure promotes a pro or antiinflammatory tumor microenvironment. Findings support the dual cytotoxic and immunomodulatory potential of snake venoms and may pave the way for the development of venom derived compounds with applications in cancer immunotherapy.

Keywords: Immunomodulatory cytokines; Snake venom; Glioblastoma.

3504 - Category: TUMOR IMMUNOLOGY (TU)

Comparative Analysis of the Cytotoxicity of Euphorbia tirucalli Latex in HCT-116 and RAW 264.7 Cell Lines

CORREIA, I.P.R.¹; FERREIRA, A.C.A.¹; LIMA, A.C.P.²; FERNANDES, A.M.S.¹; MEYER, R.¹; COSTA, R.S.¹; DA SILVA, M.B.¹. 1. UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. CENTRO UNIVERSITÁRIO JORGE AMADO, SALVADOR - BA - BRASIL.

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death in Brazil. Therefore, research into medicinal extracts as cancer treatments is necessary. Euphorbia tirucalli stands out for its latex, which is rich in bioactive compounds with antitumor and immunomodulatory potential. Investigating the effects, cellular models such as the HCT-116 tumor cell line and RAW 264.7 murine macrophages are used, representing CRC and the immune system. This study aims to perform a comparative analysis of the viability and cytotoxicity of colorectal cancer HCT-116 cells and murine RAW 264.7 after exposure to Euphorbia tirucalli latex. Cell viability was assessed using the MTT colorimetric assay. A total of 1×10⁵ cells from the HCT-116 and RAW 264.7 lines were seeded in 100 µL of DMEM medium supplemented with 10% FBS in flat-bottom 96-well plates. After incubation in a 5% CO₂ incubator for 24, 48, and 72 hours, the cells were treated with intermediate concentrations of the latex (2.5 and 200 µg/mL). For the RAW 264.7 assays, were also stimulated with LPS in addition to the latex. At the end of the incubation period, the medium was removed, MTT was added, and absorbance was read at 570 nm. The results obtained showed a broad spectrum of cytotoxic effects of the latex on HCT-116 cells, with significant inhibition of cell proliferation in a dose-dependent manner. In contrast, a significant increase in cell viability ($P < 0.05$) was observed in RAW 264.7 treated with the latex, suggesting a possible immunomodulatory effect. The in vitro MTT assay allowed the evaluation of interactions between components of Euphorbia tirucalli latex and different cell lines, revealing its cytotoxic potential against tumor cells and its possible immunomodulatory effect on immune cells. These findings pave the way for further studies on the therapeutic use of this plant extract as an antitumor and immunomodulatory agent with pharmacological potential.

Keywords: Cell viability; cytotoxicity; cancer.

3510 - Category: TUMOR IMMUNOLOGY (TU)

SETDB1 knockdown contributes to attenuated tumor progression in breast cancer

GUALBERTO, A.; SANTANA, B.; BRACCIALLI, M.; PITTELLA, F.. UNIVERSITY OF BRASILIA, BRASÍLIA - DF - BRASIL.

SETDB1 (SET Domain Bifurcated 1) is a histone methyltransferase that catalyzes H3K9 trimethylation, contributing to transcriptional silencing of tumor suppressor genes. Dysregulated SETDB1 expression has been associated with poor prognosis and aggressive phenotypes in various types of cancers, including breast carcinoma. In this study, we investigated the functional consequences of SETDB1 knockdown in MCF7 breast cancer cells, focusing on proliferation, migration, and epithelial-mesenchymal transition (EMT), processes critically involved in tumor progression and metastasis. The RNA interference-based knockdown led to a substantial reduction in the expression levels of the SETDB1 gene in MCF7 cells. As demonstrated by cell migration assays, there was a reduction in migration in SETDB1-knockdown cells. Furthermore, qRT-PCR analyses indicated a shift in the expression of the epithelial-mesenchymal transition (EMT) markers. These findings suggest that SETDB1 promotes oncogenic properties in breast cancer cells, likely through epigenetic repression of genes that restrain proliferation and inhibit EMT. Consequently, the targeting of SETDB1 emerges as a potentially efficacious strategy to modulate tumor progression and metastatic potential in luminal breast cancer. The objective of the present study is to elucidate the epigenomic landscape governed by SETDB1 and its interaction with immune-related pathways in the tumor microenvironment. Financial support: FAPDF, CNPq and UnB. Keywords: SETDB1; siRNA knockdown; Breast cancer.

3516 - Category: TUMOR IMMUNOLOGY (TU)

IN VITRO EVALUATION OF THE CYTOTOXICITY, IMMUNOMODULATION, ANTITUMOR AND ANTIOXIDANT ACTIVITIES OF MEDICINAL PLANTS FROM THE CERRADO

MORAES, S.K.¹; DESIDÉRIO, C.S.¹; BOVI, W.G.¹; LAMOGLIA, A.S.A.²; BRANDAO, S.G.¹; SILVA, A.L.C.A.E.¹. 1. UFTM - UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO, UBERABA - MG - BRASIL; 2. UFTM - UNIVERSIDADE FEDERAL TRIÂNGULO MINEIRO, UBERABA - MG - BRASIL.

The Brazilian Cerrado, renowned for its rich biodiversity, is home to numerous plant species with therapeutic potential that local communities have traditionally used. This study aimed to evaluate, in vitro, the cytotoxic, immunomodulatory, antioxidant, and antitumor properties of herbal extracts from three plants native to the Cerrado region of Minas Gerais: *Silybum marianum* (Cardo mariano), *Peritassa campestris* (Bacupari), and *Mandevilla martiana* (Infaliva). Cell viability analyses were performed using tumor cell lines (HCT116 and HeLa), non-tumor cells (Vero CCL-81), and immune cells (RAW 264.7 murine macrophages) through the resazurin assay, along with evaluation of antioxidant capacity (ABTS assay) and quantification of pro- and anti-inflammatory cytokines (IL-6 and IL-10) by ELISA. The results showed that *S. marianum* presented the most promising profile, with high tumor selectivity ($SI \geq 2$), significant reduction of IL-10 and IL-6, and intermediate antioxidant activity ($IC_{50} = 3.00$ mg/mL). *P. campestris* demonstrated strong antioxidant capacity ($IC_{50} = 0.87$ mg/mL) and selectivity toward the HeLa cell line, although with cytotoxicity in macrophages. In contrast, *M. martiana* showed lower antioxidant potential ($IC_{50} = 12.63$ mg/mL), moderate selectivity, and early toxicity in immune cells, with limited IL-10 modulation. These findings highlight the therapeutic potential of the evaluated species, especially *S. marianum*, and underscore the need for further studies to clarify their mechanisms of action, safety, and clinical applicability.

Keywords: Neoplasms; immunomodulation; medicinal plants.

2638 - Category: VACCINES (VC)

Comparison of adaptive immune responses induced by the standard and fractional doses of the 17DD-YF vaccine against yellow fever

TORRES, T.A.¹; AZEVEDO, A.C.C.²; PEREIRA, R.A.D.S.²; DOS SANTOS, L.I.³; HENRIQUES, P.M.²; ROCHA, I.A.D.C.²; OTTA, D.A.²; PERUHYPE-MAGALHÃES, V.²; CARVALHO, A.T.²; MARTINS-FILHO, O.A.²; ANTONELLI, L.R.D.V.². 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. INSTITUTO RENÉ RACHOU - FUNDAÇÃO OSWALDO CRUZ, BELO HORIZONTE - MG - BRASIL; 3. FACULDADE DE CIÊNCIAS MÉDICAS DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

The re-emergence of yellow fever (YF) urged new mass vaccination campaigns, and, in 2017, the World Health Organization approved the use of the fractional dose (FD) of the YF vaccine due to stock shortage. The FD induced similar production of neutralizing antibodies against the YF virus when compared to the standard dose (SD). Other correlates of cellular protection were described when the immunogenicity of the SD was evaluated, however, similar studies were not performed for the FD. Our aim was to compare viremia, production of antibodies and soluble mediators, and the expansion and activation of effector and memory T and B-cells after immunization with both doses of the 17DD vaccine against YF.

Study participants were selected from the population immunized with FD or SD in the vaccination campaigns in the state of São Paulo, Brazil. Blood samples were taken before, 7 and 10-15 days after vaccination. Serum samples were also taken 30-45 days after vaccination. Cells were purified from blood clots with density gradient and phenotyped by flow cytometry. The production of soluble factors was measured using a 27-plex assay, and YF-specific IgM, IgG and neutralizing antibodies were measured by ELISA and mPRN-HR assays, respectively. Viremia was measured by reverse transcription quantitative real-time PCR.

Similar levels of soluble mediators, antibodies and viremia were induced by both formulations early after immunization. However, a faster decrease in the former was observed after SD. The FD led to a sustained expansion of helper T-cells and an increased expression of activation markers on T-cells early after vaccination. Although with different kinetics, expansion of plasma cells was induced upon SD and FD immunization. Integrative analysis reveals that FD induces a more complex network involving follicular helper T cells and B-cells than SD. Our findings substantiate that FD can replace SD, inducing a protective immune response against YF.

Keywords: Yellow fever; Vaccine; Fractional dose.

2641 - Category: VACCINES (VC)

Therapeutic potential of a TS/ASP-2 chimeric protein in experimental chronic Chagas cardiomyopathy.

PIAU, B.C.¹; DE CASTRO, N.S.²; MACHADO, C.E.C.³; ASSIS, B.R.D.³; FERREIRA, R.¹; GOMES, I.P.³; GUIMARÃES, A.C.G.³; FIORINI, A.C.³; CRUZ, J.D.S.¹; FERNANDES, A.P.³; DE CASTRO, J.T.³; TEIXEIRA, S.M.R.³; GAZZINELLI, R.T.³. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS - UFMG, BELO HORIZONTE - MG - BRASIL; 2. CENTRO DE TECNOLOGIA E DIAGNÓSTICO EM VACINAS - CT, BELO HORIZONTE - MG - BRASIL; 3. CENTRO DE TECNOLOGIA E DIAGNÓSTICO EM VACINAS - CT VACINAS, BELO HORIZONTE - MG - BRASIL.

Chagas disease (CD) is caused by the protozoan *Trypanosoma cruzi* and until nowadays there is no vaccine available. Benznidazole (BZ), the primary drug for CD, has limited efficacy in the chronic phase and may lead to several adverse effects. Therefore, vaccines are a promising alternative to prevent or treat the disease. Our group developed a chimeric protein, TRASP, combining regions of the highly immunogenic proteins Trans-sialidase (TS) and Amastigote Surface Protein-2 (ASP-2). In a previous study, a prophylactic immunization of mice and dogs was effective in inducing a protective immune response against infection. Here, we evaluate the potential of TRASP as a therapeutic vaccine. For that, C57BL/6 mice were infected with 100 trypomastigotes of the myotropic Colombian strain, and after disease chronification, animals were treated with TRASP + adjuvants (Adj, Poly-ICLC or MF59), associated or not with a quarter dose of BZ (25 mg/kg) for 30 days. The groups with associated treatments received the first dose of TRASP + Adj either 20 days before (TRASP + Adj + BZ) or 7 days after (BZ + TRASP + Adj) the beginning of BZ treatment. Control groups received only PBS, Adj, or BZ. Cellular immune response was analyzed at 200 days post infection (DPI) by *in vitro* stimulation of splenocytes. It was observed a predominance of IFN- γ over IL-10 and IL-17, especially in the TRASP + Adj + BZ, indicating a Th1 recall response. Regarding improvement in symptomatology, we detected lower levels of creatine kinase MB (CK-MB) in mice administered with TRASP + Adj + BZ, and they also present better electrocardiogram parameters, similar to non-infected mice. Taken together, our data show that this is a promising therapeutic vaccine, particularly when administered before and in combination with BZ treatment. This work was supported by Fundação Romeu Cançado, CAPES, CNPq, FAPEMIG, and INCTV.

Keywords: Chimeric protein vaccine; Therapeutic protocol; Chronic Chagas Cardiomyopathy.

2642 - Category: VACCINES (VC)

Evaluation of the immune response against the yellow fever virus (vaccine and wild-type strains) in vaccinated individuals.

BRITO, D.M.D.S.; ALMEIDA, L.T.; SILVA, M.F.A.S.E.; GONÇALVES, A.P.; CAMPI-AZEVEDO, A.C.; DE CARVALHO, A.T.; MARTINS-FILHO, O.A.; ALVES, P.A.. INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Yellow fever (YF) is an acute infectious disease caused by *Orthoflavivirus flavi*, also known as yellow fever virus (YFV). Recognized since the 17th century, YF was the first identified hemorrhagic fever and has been responsible for multiple outbreaks, significantly impacting global public health. Currently, no specific antiviral treatment is available, so the live-attenuated vaccines remain the most effective tools for YF prevention, having averted millions of deaths. These vaccines are derived from the Asibi strain, a wild-type (WT) YFV isolated from a Ghanaian patient. The attenuated strain was obtained through serial passages in cell cultures with limited permissiveness to viral multiplication. Previous studies suggest that the humoral immune response induced by vaccination differs from that elicited by natural infection. Patients infected during the 2017–2018 Brazilian outbreak exhibited higher neutralizing antibody (nAb) titers against WT YFV than against 17DD, indicating a more robust homotypic response. This study aimed to evaluate the humoral response – homotypic and heterotypic – in individuals vaccinated with 17DD. Serum samples were obtained from healthy adults (n=23) previously enrolled in a 2018 immunogenicity study in São Paulo, Brazil. Paired serum samples were collected before vaccination (day 0) and 30–45 days post-vaccination. nAb titers were assessed using Plaque Reduction Neutralization Tests (PRNT) against both vaccine and WT YFV strains. Additionally, cytokine and chemokine levels were quantified via a Luminex® 48-plex assay. Pre-vaccination samples showed no neutralizing activity. Post-vaccination samples revealed higher geometric mean titers against 17DD compared to WT YFV. Cytokine and chemokine levels showed no significant differences between vaccinated and non-vaccinated individuals. These findings enhance our understanding of YF vaccine-induced humoral and cellular responses and support future epidemiological and public health strategies.

Keywords: Yellow fever; Immune response; PRNT.

2649 - Category: VACCINES (VC)

Age-Dependent Duration of Immune Response to 17DD Yellow Fever Vaccine in Non-Endemic Areas

ROCHA, I.A.D.C.¹; REIS, L.R.²; LEMOS, P.B.D.M.³; DE LISBOA, M.F.S.¹; TORRES, T.A.⁴; FERROCO, C.L.D.V.⁵; MAIA, M.D.L.D.S.⁵; PERUHYE-MAGALHÃES, V.¹; CARVALHO, A.T.¹; CAMPI-AZEVEDO, A.C.¹; MARTINS-FILHO, O.A.¹. 1. INSTITUTO RENÉ RACHOU - FIOCRUZ-MINAS, BELO HORIZONTE - MG - BRASIL; 2. CLEVELAND CLINIC FLORIDA RESEARCH & INNOVATION CENTER, PORT ST. LUCIE - ESTADOS UNIDOS DA AMERICA; 3. UNIVERSIDADE FEDERAL DE UBERLÂNDIA, UBERLÂNDIA - MG - BRASIL; 4. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 5. BIO-MANGUINHOS, RIO DE JANEIRO - RJ - BRASIL.

Yellow fever (YF) remains a serious global public health concern, particularly due to the expanding transmission areas of the YF virus. Since vaccination is the most effective measure of protection, understanding the duration of immune response elicited by the 17DD YF vaccine is essential for guiding vaccination strategies in Brazil, especially in non-endemic regions. In this context, we evaluated humoral and cellular immune responses in a cohort of residents of non-endemic areas, including children aged 9^{mths}–2^{yrs}, 3–4 ^{yrs} and adults aged 18–49 ^{yrs}, at multiple time-points: pre-vaccination (D0), 30–45 days (D30–45), 1- (1^{yr}) and 4-years (4^{yrs}) post-vaccination. Neutralizing antibody titers and seropositivity rates were assessed, along with phenotypic analysis of CD4⁺, CD8⁺ T-cells and B-cells following *in vitro* 17DD antigen recall. Our results showed higher neutralizing antibody titers and seropositivity rates in adults and older children at D30–45 and 1^{yr}, with significant declines at 4^{yrs} in both child subgroups. The phenotypic analysis demonstrated that CD4⁺ T-cell response displays an early increase in early effector subset in all age groups along with persistence increase of effector memory cells in younger children. In addition, CD8⁺ T-cell memory subsets showed a dynamic profile, with a notable decrease of effector and central memory cells in 9^{mths}–2^{yrs} group, as well as a sustained IFN-γ-mediated memory cells up to 1^{yr}, followed by a decline in 9^{mths}–2^{yrs} group at 4^{yrs}. Memory B-cells also increased shortly after vaccination but waned in young children by 4^{yrs}. These findings highlight a marked disparity in the duration of vaccine-induced immunity between age groups, with rapid immune waning in children aged 9^{mths}–2^{yrs}. In the context of YF resurgence, our data underscores the need to review booster policies and adapt immunization schedules to better protect vulnerable populations.

Keywords: Yellow fever vaccine; Neutralizing antibodies; T- and B-cell response.

2658 - Category: VACCINES (VC)

DEVELOPMENT AND EVALUATION OF THE EFFICACY OF DNA VACCINES AGAINST THE OMICRON VARIANT AND ITS XBB.1.5 AND JN.1 SUBVARIANTS IN EXPERIMENTAL MODELS

OLIVEIRA, E.R.; MACHADO, C.E.C.; DE AZEVEDO, P.O.; GAZZINELLI, I.H.; FIGUEIREDO, M.M.; DORO, D.; DE SOUZA, N.S.H.; GAZZINELLI, R.T.. INSTITUTO RENE RACHOU, BELO HORIZONTE - MG - BRASIL.

Over the past few years, SARS-CoV-2 has demonstrated remarkable adaptability, as evidenced by the emergence of multiple variants. This evolutionary pressure underscores the constant need to develop new, effective vaccines against emerging variants. Therefore, this study proposes the development of a DNA vaccine encoding the Spike protein of the XBB.1.5 subvariant (pCTV-XBB), evaluated in experimental models. The constructed plasmid successfully expressed the Spike protein in vitro, and immunization with two doses of pCTV-XBB induced high titers of binding and neutralizing antibodies, along with activation of memory T cells. K18-hACE2 mice and Syrian hamsters were immunized to evaluate the plasmid's protective efficacy against XBB.1.5 challenge, resulting in reduced viral loads in both the lungs and nasal lavage, as well as preservation of lung architecture in histopathological analyses. To elucidate the immunological mechanisms involved in protection, serum transfer assays and experiments using B cell-deficient ACE2-transgenic mice (BKO/K18-hACE2) were conducted. Neutralizing antibodies induced by immunization were essential for protection against infection, although cellular responses also contributed to viral control. Additionally, immunization with pCTV-XBB elicited cross-immunity against the JN.1 subvariant, as indicated by the reduction in pulmonary viral load. Thus, the results obtained in this study, along with the mutational potential of SARS-CoV-2, highlight DNA vaccines as a versatile and adaptable platform for facing new variants of SARS-CoV-2. The possibility of rapid updating of the antigenic coding sequence, combined with the stability of the formulation and a favorable safety profile, makes the DNA platform promising for the development of vaccines against current and future SARS-CoV-2 variants.

Keywords: DNA vaccines;XBB;1;5 subvariant;COVID-19.

2672 - Category: VACCINES (VC)

mRNA vaccine protects mice against highly pathogenic avian influenza H5N1 virus

SILVA, G.D.A.¹; FAUSTINO, L.P.¹; CALDAS, G.D.A.B.²; FERNANDES, R.S.²; MOLINA, D.A.²; FIGUEIREDO, M.M.²; DURIGON, E.³; TEIXEIRA, S.M.R.²; DE SOUZA, N.S.H.¹; GAZZINELLI, R.T.². 1. INSTITUTO RENÉ RACHOU, BELO HORIZONTE - MG - BRASIL; 2. CENTRO DE TECNOLOGIA DE VACINAS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Viral surface glycoproteins such as hemagglutinin and neuraminidase undergo constant mutations, resulting in reduced vaccine efficacy. In contrast, nucleoprotein (NP) is highly conserved among different strains and immunogenic for T lymphocytes. mRNA vaccines have emerged as an important vaccine platform due to the ease of updating to new antigens. Our study aimed to develop a mRNA vaccine for influenza virus based on NP from the isolate A/PR/8/34 (H1N1). The sequence encoding the NP gene was cloned into a pCDNA plasmid containing optimized 5' and 3' UTR sequences and poly-A tail. The in vitro synthesis of mRNA was performed in the presence of N1-Methyl-Pseudouridine and a cap analogue. In vitro expression of the NP protein was verified by immunofluorescence and western blot. C57BL/6 mice were immunized with two doses of LNP-NP (NP mRNA encapsulated in a lipid nanoparticle) 21 days apart. Antibody titers were measured before the second dose and 30 days later. T-cell response was also evaluated 30 days after the second dose. Mice were challenged with 10⁷ PFU of H5N1 (2.3.4.4b clade) for the evaluation of body weight, survival, viral load and histopathology of lung samples. The construction of the plasmid was confirmed by digestion and sequencing. Cells transfected with mRNA-NP were able to express the NP in vitro. Mice immunized with LNP-NP developed high titers of total IgG in their sera and BALF samples. A high titer of IgG1 and IgG2c was also observed in sera samples. Splenocytes from mice immunized with LNP-NP secreted high levels of IFN-γ in response to restimulation in vitro with recombinant NP. All mice immunized and challenged with the virus maintained their body weight and survived. In addition, lower viral loads and maintenance of lung architecture were observed in mice immunized and challenged. Our results indicate that LNP-NP is a promising vaccine that could be used in the case of a future endemic or pandemic.

Financial support: CNPq, CAPES, FAPEMIG

Keywords: H5N1;Nucleoprotein;mRNA.

2674 - Category: VACCINES (VC)

PRE-CLINICAL STUDY OF VACCINE CANDIDATES AGAINST ZIKA VIRUS, USING RECOMBINANT VIRAL ENVELOPE PROTEIN, FUNCTIONALIZED OR NOT IN GOLD NANORODS

GODINHO, I.P.¹; MORAES, T.D.F.S.¹; ROCHA, V.D.M.¹; SALOMÃO, L.F.C.¹; BAGNO, F.F.²; MARTINS, E.M.D.N.³; DOS REIS, J.G.A.C.¹; BARBOSA-STANCIOLI, E.F.¹; DA FONSECA, F.G.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS - LABORATÓRIO DE VIROLOGIA BÁSICA E APLICADA, DEPARTAMENTO DE MICROBIOLOGIA, BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS - CT VACINAS - CENTRO NACIONAL DE TECNOLOGIA EM VACINAS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS - CDTN - CENTRO DE DESENVOLVIMENTO DA TECNOLOGIA NUCLEAR, BELO HORIZONTE - MG - BRASIL.

The association of *Orthoflavivirus zikaense* (ZIKV) with microcephaly and neurological complications turned this arbovirus into a global public health problem in the last decade. With the biotechnological advancement of new approaches in the development of efficient vaccines, nanotechnology has stood out. This work presents the evaluation of the cellular and humoral immune response developed by two experimental vaccines developed (ZIKV-E and ZIKV-E+GNR) against ZIKV in the C57BL/6 model, followed by a challenge with ZIKV in previously immunized pregnant female C57BL/6 mice, in order to observe possible alterations in the prenatal neurodevelopment of the fetuses, verifying vertical transmission as reported in the literature. After immunization of the mice, high levels of anti-ZIKV neutralizing antibodies were detected. Furthermore, experimental vaccines were able to induce the activation of immune system cells, increasing IFN- γ secretion in CD8⁺ CD107a⁺ cells and a tendency to increase IFN- γ and TNF secreted by CD8⁺ effector and central lymphocytes in memory phenotype cells. These results were observed mainly in the group immunized with ZIKV-E+GNR. Fetal viability has been shown to be affected in an infection scenario without the vaccination (65,44%), with a greater number of resorptions compared to the number of fetuses in the offspring in a scenario without the challenge (98,33%). Surprisingly, the animals immunized with ZIKV-E+GNR and subsequently challenged had a fetal viability value of (94,78%), similar to the animals in the non-challenged group, suggesting that the experimental vaccines were satisfactorily protective.

Keywords: Zika virus; Nanotechnology; Subunit vaccines.

2705 - Category: VACCINES (VC)

Adipokines and antibody response in individuals with physical activity and sedentary behaviour following COVID-19 vaccination.

OLIVEIRA, L.A.R.¹; MARTINS, L.C.²; DESIDÉRIO, C.S.³; DE OLIVEIRA, C.J.F.³; JÚNIOR, V.R.³; BORTOLINI, M.J.S.²; TAKETOMI, E.A.²; RESENDE, R.D.O.². 1. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. UFU, UBERLÂNDIA - MG - BRASIL; 3. UFTM, UBERABA - MG - BRASIL.

Sedentary behavior may be associated to chronic diseases, including obesity, type 2 diabetes, and cardiovascular diseases, leading to immune system dysregulation. Adipokines, released by adipocytes and immune cells in the adipose tissue, play a pivotal role in modulating inflammatory responses, which may be mediated by physical exercise. This study investigated the influence of physical exercise on antibody response and adipokine profile following SARS-CoV-2 vaccination. Sera samples from 135 participants selected by strength tests and International Physical Activity Questionnaire (IPAQ) were analyzed for quantification of anti-S IgG, neutralizing antibodies (NAb), adiponectin, leptin, resistin, IL-10, and TNF- α . No correlation was found between NAb and muscle strength. Notably, women demonstrated higher levels of leptin and resistin compared to men, regardless of lifestyle. However, adiponectin levels were significantly higher in women compared to men (5,099 pg/mL vs. 3,938 pg/mL, $p < 0.01$). Furthermore, women exhibited a greater production of IgG in relation to force, taken by lower FBMI/IgG ratio (0.52), compared to men (0.76; $p < 0.0001$), suggesting a potential gender-related differences in the immune response. These findings emphasize the significant impact of physical exercise and gender on COVID-19 vaccine-induced immunity, highlighting the importance of counselling the physical activity for vaccine efficacy and additional vaccination strategies based on populational lifestyle.

Keywords: COVID-19; vaccine; physical activity.

2734 - Category: VACCINES (VC)

Gut-adjacent visceral adipose compartments as a reservoir for oral vaccine-induced specific T cells

DA SILVA, G.W.¹; GONÇALVES, L.M.¹; OLIVEIRA, B.D.C.¹; RODRIGUES, G.M.B.¹; DE ASSIS, J.B.¹; ALVES, C.B.¹; DE ABREU, A.M.¹; DA CONCEIÇÃO, L.B.¹; CARDOSO, A.¹; LIMA, G.D.M.¹; COELHO, M.P.S.¹; CAMARGO, L.¹; GOMES, E.M.²; NORTON, E.³; DE ARAUJO, M.V.¹; DA FONSECA, D.M.¹. 1. ICB-USP / INCT MUCOSA AND SKIN, SÃO PAULO - SP - BRASIL; 2. ICB-USP, SÃO PAULO - SP - BRASIL; 3. TULANE UNIVERSITY, NEW ORLEANS - ESTADOS UNIDOS DA AMERICA.

The intestinal mucosa is one of the largest areas of the body exposed to the external environment. It operates in the absorption of nutrients, immunity against pathogens and sustains immunological tolerance. Not only the gut-associated lymphoid tissue, but also other gut-adjacent organs, such as visceral adipose tissues (VAT), ensure homeostasis in mucosal tissues. VAT, such as mesentery, has already been described as an infection-induced memory T cell reservoir. Still, it is not clear whether these tissues could sustain mucosal vaccine-induced memory cells. Our goal is to elucidate the contribution of VAT, particularly the mesentery and omentum, as major reservoirs of oral vaccine-elicited memory cells. We tested an experimental oral vaccine platform performed in a prime-boost system: a double mutant heat-labile toxin (LTR192G/L211A) as an adjuvant, together with the protein ovalbumin or with heat-killed *Salmonella typhimurium* SL1344 as the experimental antigen. Mice were immunized with the oral vaccine by gavage. We performed flow cytometry and ELISA assay for cell and antibody characterization. The oral immunization induced antigen-specific IgG1 and IgG2a antibodies in the serum and antigen-specific IgA in the intestinal lavage. It was also efficient in promoting the activation of cellular adaptive responses in the gut lamina propria and protected mice against bacterial challenge. Oral adjuvanted vaccination elicited specific T cells not only in the small intestine lamina propria but also in the omentum and mesentery of the immunized mice. These results indicate the use of dmLT as an adjuvant for oral vaccines and also suggest gut-adjacent compartments, especially the VAT, harboring protective cells prone to act during oral infection episodes. Next, we intend to assess the contribution of VAT to support memory responses when the intestinal mucosa is damaged, such as during colitis, obesity, or environmental enteropathy.

Keywords: adipose;memory;adjuvant.

2742 - Category: VACCINES (VC)

Response to COVID-19 vaccination in neurotypical children and children with Down syndrome

DE OLIVEIRA, M.C.¹; FAMILIAR-MACEDO, D.¹; ZANGIROLAMI, L.A.¹; FLINTZ, R.¹; CARVALHO, F.R.²; VIANNA, R.A.²; CARDOSO, C.A.A.²; DA SILVA, R.T.B.³; BAUMBLATT, A.P.³; DE-OLIVEIRA-PINTO, L.M.¹. 1. FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL FLUMINENSE (UFF), NITERÓI - RJ - BRASIL; 3. UNIVERSIDADE ESTADUAL DO RIO DE JANEIRO (UERJ), RIO DE JANEIRO - RJ - BRASIL.

Children with Down Syndrome (DS) infected with SARS-CoV-2 are susceptible to respiratory failure, multisystem inflammatory syndrome and require longer periods of hospitalization and mechanical ventilation. Vaccines against SARS-CoV-2 were registered for people with DS, but few studies were made with DS children. This study aims to evaluate humoral immune response (IgA, IgG and neutralizing IgG) to SARS-CoV-2 vaccination in children with DS compared to children without DS. Blood samples were collected in 2021-2023 from 56 children between 1-15 years old. The control group included 22 children (13 vaccinated, 9 unvaccinated) and DS group included 34 (13 vaccinated, 21 unvaccinated). Anti-SARS-CoV-2 IgA and IgG assays and a viral neutralization test for neutralizing IgG were performed by ELISA. Approximately 70% of children received the BNT162b2 (Pfizer) vaccine and 30% had Coronavac (Butantan). Vaccinated children in control group had higher levels of anti-SARS-CoV-2 IgA than unvaccinated. DS children had similar IgA levels before and after vaccination. All vaccinated children showed increased levels of anti-SARS-CoV-2 IgG and neutralizing IgG compared with unvaccinated children. Additional analyses comparing natural infection and vaccination showed an increase in specific IgA levels in vaccinated control children compared with those naturally infected, but no difference was found between vaccinated and naturally infected DS children. Specific IgG levels increased with vaccination in all children compared with those naturally infected. However, only vaccinated control children showed an increase in neutralizing IgG compared with those naturally infected. In conclusion, the COVID-19 vaccine induced specific and neutralizing IgG in all children, but specific IgA induction was observed only in the control group children. Our data indicate that vaccination should be encouraged, and further studies are needed to clarify the low IgA response in children with DS.

Keywords: SARS-CoV-2;Down syndrome;Antibodies.

2743 - Category: VACCINES (VC)

Antigenicity and Immunogenicity of PvRMC-1, a novel chimeric multistage vaccine candidate against *Plasmodium vivax*

MATOS, A.D.S.¹; SOARES, I.F.¹; BAPTISTA, B.D.O.¹; DE SOUZA, H.A.D.S.¹; CHAVES, L.B.¹; PERCE-DA-SILVA, D.D.S.²; RICCIO, E.K.P.¹; ALBRECHT, L.³; RODRIGUES-DA-SILVA, R.N.¹; LOPEZ-CAMACHO, C.⁴; RODOLPHI, C.M.¹; DONASSOLO, R.A.³; BOM, A.P.D.A.⁵; NEVES, P.C.D.C.⁵; CONTE, F.D.P.⁵; TOTINO, P.R.R.¹; DANIEL-RIBEIRO, C.T.¹; LIMA JUNIOR, J.D.C.¹. 1. FIOCRUZ/IOC, RIO DE JANEIRO - RJ - BRASIL; 2. CENTRO UNIVERSITÁRIO ARTHUR SÁ EARP NETO/FACULDADE DE MEDICINA DE PETRÓPOLIS (UNIFASE/FMP), PETRÓPOLIS - RJ - BRASIL; 3. FIOCRUZ/INSTITUTO CARLOS CHAGAS, CURITIBA - PR - BRASIL; 4. THE JENNER INSTITUTE, UNIVERSITY OF OXFORD, OXFORD - INGLATERRA; 5. FIOCRUZ/BIOMANGUINHOS, RIO DE JANEIRO - RJ - BRASIL.

In the Americas, *P. vivax* is the predominant causative species of malaria, a debilitating and economically significant disease. Due to the complexity of the malaria parasite life cycle, a vaccine formulation with multiple antigens expressed in various parasite stages may represent an effective approach. Based on this, we designed and constructed a chimeric recombinant protein, PvRMC-1, composed by PvCyRPA, an erythrocyte candidate, PvCelTOS, a proerythrocyte candidate and epitopes of Pvs25, of sexual stage. The antigenicity was assessed by IgM and IgG (total and subclass) ELISA in 301 naturally exposed individuals from the Brazilian Amazon. The recombinant protein was recognized by IgG (54%) and IgM (40%) antibodies in the studied individuals, with a predominant cytophilic response mediated by IgG1 (70%) and IgG3 (69%), suggesting the success in construction and confirms the antigenicity of this antigen. Then, we evaluated the immunogenicity of PvRMC-1 formulated in three distinct adjuvants: Stimune, AddaVax or Aluminum hydroxide (Al(OH)₃) in BALB/c mice. Our results suggested that the chimeric protein were capable to generate humoral response across all three formulations for full-length PvRMC-1 and all linear B-cell epitopes individually, with high antibody titers up to 136 days after the initial immunization. Moreover, for the evaluation of cellular response accessed by ELISPOT, mice's splenocytes were activated, producing IFN- γ in response to PvCelTOS and PvCyRPA peptide epitopes, affirming T-cell epitopes in the antigen. Comparing the adjuvants, aluminum hydroxide showed notable cellular response, Stimune and Addavax induced a more comprehensive immune response, encompassing both cellular and humoral components. Thus, our findings indicate that PvRMC-1 was successfully expressed, targeted by natural antibodies and would be a promising multistage vaccine candidate that could advance to further preclinical studies.

Keywords: Chimeric Protein; *Plasmodium vivax*; Malaria Vaccine.

2753 - Category: VACCINES (VC)

Immunogenic Potential of a Chimeric Vaccine against *Streptococcus pneumoniae* in an Animal Model

DOS SANTOS, J.S.C.; DE REZENDE, S.A.F.; DE PAULA, K.G.; RAMOS, K.K.S.; CUNHA, L.M.; DE CAMARGO, D.R.A.; LECLERCQ, S.Y.. FUNDAÇÃO EZEQUIEL DIAS, BELO HORIZONTE - MG - BRASIL.

Streptococcus pneumoniae is a gram-positive bacterium and the etiological agent of several diseases, such as pneumonia, meningitis, bacteremia, and others. Pneumococcal diseases can be prevented by vaccination; however, currently available vaccines have some disadvantages, especially due to the high variability of the capsule of streptococcal strains, which is their main virulence factor, and there are currently more than 100 distinct serotypes. Thus, an effective prevention strategy continues to be the focus of much research. Through bioinformatics tools, a chimeric protein was constructed from immunogenic epitopes of conserved antigens of *S. pneumoniae*. Therefore, the objective of this study is to evaluate the protective potential of this chimeric protein in order to obtain a vaccine with greater vaccination coverage. For this purpose, a sepsis model was used in *Swiss Webster* mice, which were immunized with three doses of the vaccine composed of the recombinant chimeric protein. ELISA assays were performed to evaluate the humoral response. The animals were challenged with *S. pneumoniae* and the mortality rate was monitored. The results suggest immunogenicity of the chimeric protein, since it induced a response with high rates of total IgG and IgG1. Based on the results obtained to date, the vaccine composed of the chimeric protein was immunogenic; however, it did not protect the animals against the challenge with *S. pneumoniae*. New ELISA assays are being performed to determine the IgG2b titer, since the IgG2a titer was not high. In addition, new tests will be performed using other bacterial strains and other formulations so that we can determine the real efficacy of the chimeric protein.

Keywords: *Streptococcus pneumoniae*; pneumonia; chimeric recombinant protein.

2760 - Category: VACCINES (VC)

Evaluation of the Immunogenic Potential of a Vaccine Against *Mycoplasma pneumoniae* in an Animal Model

SALOMON, L.S.¹; CUNHA, L.M.²; RODRIGUES, T.C.V.³; AZEVEDO, V.A.D.C.³; SILVA, L.M.²; DOS SANTOS, J.S.C.².
1. FUNDAÇÃO EZEQUIEL DIAS - PROGRAMA DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA, BELO HORIZONTE - MG - BRASIL; 2. FUNDAÇÃO EZEQUIEL DIAS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Mycoplasma pneumoniae is a bacterium with unique morphological and metabolic characteristics, and is one of the main pathogens associated with community-acquired pneumonia (CAP). It poses a significant public health challenge due to difficulties in diagnosis and increasing antibiotic resistance. Currently, there are no licensed vaccines against this pathogen, highlighting the urgent need for the development of immunoprotective strategies.

In this context, the present study aims to evaluate the immunogenic potential of a chimeric multi-epitope protein, **mpme-VAC/STV-1**, developed through reverse vaccinology and immunoinformatics. The protein was designed based on selected epitopes from five conserved proteins of the *M. pneumoniae* genome and will be used in a vaccine formulation. For this purpose, 32 Swiss Webster mice will be distributed into four groups: (1) PBS + nanoemulsion; (2) PBS; (3) 20 µg of the recombinant chimeric protein + nanoemulsion; and (4) 20 µg of the recombinant chimeric protein. The animals will receive three intramuscular immunizations at 15-day intervals to assess the production of specific antibodies and the expression of cytokines IFN-γ, TNF-α, IL-17, IL-6, and IL-12.

The chimeric gene was cloned into the pET-28(+) expression vector and used to transform *Escherichia coli* BL21(DE3) for recombinant protein expression. Transformed colonies underwent plasmid DNA extraction, and the presence of the chimeric gene was confirmed by enzymatic digestion with **BamHI** and **EcoRI**, yielding a fragment compatible with the expected size (~1740 bp), as well as by PCR using T7 promoter primers, which produced an amplicon of approximately 2000 bp.

The results of this study may contribute to advancing the development of a vaccine against *M. pneumoniae*, offering an alternative for the prevention of CAP and reducing reliance on antimicrobial therapies.

Keywords: *Mycoplasma pneumoniae*; community-acquired pneumonia; vaccine.

2776 - Category: VACCINES (VC)

Evaluation of T Cell Responses Induced by α-Type 1 Dendritic Cells Loaded with Topologically Relevant HIV-1 Peptides

ORTEGA, M.M.; DA SILVA, L.T.; TIYO, B.T.; MOTA, S.D.J.; SCHMITZ, G.J.H.; SUMIDA, T.M.O.; DUARTE, A.J.D.S..
FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Background: Despite the significant progress achieved with antiretroviral therapy (ART), a functional cure for HIV-1 remains elusive due to viral latency and immune dysfunction. Among several immunotherapies under studies, dendritic-cell based approaches, particularly those employing autologous monocyte-derived DCs (moDCs) pulsed with conserved HIV-1 peptides, offers a promising approach to restore effective antiviral immune responses. **Objectives:** This study aimed to evaluate the capacity of moDCs, polarized into alpha-type 1 DCs (αDC1) and pulsed with highly conserved HIV-1 peptides, to stimulate HIV-specific CD4+ and CD8 + T cells responses in vitro, using samples from a cohort of chronically HIV-1 infected individuals under long-term ART. **Methods:** Peripheral blood mononuclear cells (PBMCs) were obtained from 23 HIV-1-infected individuals. Eighteen peptide pairs derived from HIV-1 Gag and Pol regions were used for pre-selection based on their immunogenic potential to induce CD4+ and CD8 + T responses. αDC1s were cultured for five days, pulsed with seven pre-selected peptides and after 2 hours, cells were matured with a pro-inflammatory cytokine cocktail for two additional days. On day seven, αDC1s were co-cultured with autologous T cells for six days. In the final 24 hours, cells were re-stimulated with the same previously peptides. Intracellular IFN-γ production of CD4 + and CD8 + T cells was assessed by flow cytometry. **Results:** When pulsed with previously selected HIV-1 peptide, αDC1 induced a specific IFN-γ production, mainly in CD8 + T cells. These findings highlight the functional capacity of dendritic cells pulsed with conserved peptides to elicit specific cellular responses. **Conclusion:** αDC1-based stimulation with conserved HIV-1 peptides effectively reactivates HIV-specific T cell responses, supporting its potential as a therapeutic immunization in chronic HIV-1 infection.

Keywords: Immunotherapy ;Dendritic Cells;T Cells Responses.

2778 - Category: VACCINES (VC)

Optimizing mRNA Vaccine Development via Nucleotide Modifications: Balancing Stability, Immune Activation, and Antigen Expression

BERNARDINO, T.C.U.; DE SOUZA, R.S.; VIVEIROS, D.D.; FRANÇA, T.D.S.; DE CARVALHO, C.; NEVES, P.C.D.C.; BOM, A.P.D.A.; JÚNIOR, H.C.D.S.. BIO-MANGUINHOS/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

mRNA technology has significantly impacted vaccine development by enabling rapid and flexible production. However, important challenges remain, particularly related to mRNA stability and activation of innate immune responses, which may compromise vaccine performance and safety. In this context, nucleotide modification has emerged as a promising approach to reduce immunogenicity while enhancing protein expression. To investigate this, modified nucleotides—including four uridine (UTP-1, UTP-2, UTP-3, UTP-4), cytidine (CTP-1), and adenosine (ATP-1) analogs—were incorporated during in vitro transcription into an mRNA construct encoding the SARS-CoV-2 Spike protein fused to GFP. Subsequently, double-stranded RNA (dsRNA) levels were measured using an ELISA-based assay, and protein expression was evaluated by flow cytometry following transfection into HEK-293T cells. The analysis revealed elevated dsRNA levels in mRNAs containing unmodified uridine and UTP-3, suggesting a higher potential to trigger innate immune responses. In contrast, UTP-1, UTP-2, and CTP-1 were associated with lower dsRNA levels, suggesting reduced immunostimulatory potential. Regarding protein expression, mRNAs containing unmodified uridine, UTP-1, UTP-2, and CTP-1 showed increased antigen expression, whereas UTP-3 and ATP-1 led to significantly lower levels. Taken together, these differences in dsRNA content and protein expression indicate that specific nucleotide modifications may affect essential characteristics of mRNA. These findings highlight how targeted modifications can modulate immune responses and translation efficiency, paving the way for safer and more effective mRNA vaccines.

Keywords: mRNA-based vaccines;innate immune responses;nucleotide modifications.

2779 - Category: VACCINES (VC)

Immunization with Kharon-deficient *Leishmania mexicana* Induces Cross Protection Against challenge with *Leishmania braziliensis* in a Preclinical ModelCOSTA, T.D.S.¹; DE SANTANA, M.B.R.¹; BORBA, P.¹; DA SILVA, L.O.¹; NOGUEIRA, P.M.²; GARCIA, M.A.D.N.³; MOREIRA, P.O.L.²; MORETTI, N.S.⁴; NETO, R.L.D.M.²; DE OLIVEIRA, C.I.¹. 1. IGM FIOCRUZ, SALVADOR - BA - BRASIL; 2. IRR FIOCRUZ, BELO HORIZONTE - MG - BRASIL; 3. LBMP, DEPTO. DE MICROBIOLOGIA, IMUNOLOGIA E PARASITOLOGIA, UNIFESP, SÃO PAULO - SP - BRASIL; 4. FACULTÉ DE MÉDECINE VÉTÉRINAIRE, UNIVERSITÉ DE MONTRÉAL, SAINT HYACINTHE, QUÉBEC - CANADA.

The use of live attenuated parasites of the genus *Leishmania* as vaccine candidates has been resumed and widely studied, as they mimic natural infections without causing the disease, allowing interaction with essential antigens in the development of immunity. Previous studies have shown that deletion of the kharon1 gene results in defects in the development of the amastigote form, leading to intracellular death and rendering the parasites incapable of sustaining the infection. In this work, we evaluated the ability of a Kharon-deficient *L. mexicana* strain ($\Delta Lmexkh1$) to induce cross-protection against *L. braziliensis*, Brazil's leading etiological agent of Cutaneous Leishmaniasis. BALB/c mice were immunized intradermally with $\Delta Lmexkh1$ parasites and monitored over time. No lesions were observed, confirming that $\Delta Lmexkh1$ parasites are safe and non-pathogenic. Cytokine profiling in mice immunized with $\Delta Lmexkh1$ showed elevated levels of IFN- γ in draining lymph nodes compared to mice infected with wild-type *L. mexicana*, along with lower levels of inflammatory cytokines IL-4, indicating the development of a dominant Th-1 immune response following immunization. To evaluate vaccine efficacy, immunized mice were challenged with *L. braziliensis*, four weeks after inoculation of $\Delta Lmexkh1$. Challenged mice did not develop lesions and showed a significantly reduced parasite burden compared to non-immunized controls, indicating strong cross-protection. Immune profiling of draining lymph nodes post-challenge revealed that challenged mice produced significantly higher levels of IFN- γ and lower levels of TNF- α , IL-4, and IL-6 which can be correlated with the lower parasite burden. These findings reinforce the potential of the $\Delta Lmexkh1$ strain as a vaccine candidate against different *Leishmania* species.

Keywords: Live attenuated *Leishmania* vaccine;Kharon;Immune response.

2780 - Category: VACCINES (VC)

Immunopathological and Microbiological Evaluation of In Ovo *E. coli* Vaccination in Broilers Challenged with Avian Pathogenic *Escherichia coli*

BASTOS, A.P.¹; OGONCZYK, D.²; HAACH, V.²; BETBEDER, D.². 1. EMBRAPA SUINOS E AVES, CONCÓRDIA - SC - BRASIL; 2. VAXINANO, LOOS - FRANÇA.

This study aimed to evaluate the immunogenicity and protective potential of a nanoparticle-based vaccine containing recombinant antigens from avian pathogenic *Escherichia coli* (APEC) administered either in-ovo or via mucosal route in broiler chickens. A total of 210 Ross 308 embryonated eggs were randomly allocated into seven groups (n=30). Chicks were vaccinated in-ovo at day 18 of incubation or by ocular/nasal route on the first day post-hatch. Vaccinated groups were challenged with a virulent APEC strain (Embrapa 23345), while non-challenged and challenged non-vaccinated groups served as negative and positive controls. Key parameters evaluated included phagocytic activity of monocytes and heterophils, intestinal permeability, anti-*E. coli* IgA and IgY levels in serum and mucosal secretions, gene expression of cytokines (IL-1 β , IFN γ , IL-4, IL-10) in ileum and lung, bacterial colonization of organs, and histopathological lesions. Samples were collected at 20 and 27 days post-hatch to assess lesions in liver, heart, lungs, and ileum, quantify bacterial load via qPCR, and determine the frequency of virulence genes (*iroN*, *ompT*, *hlyF*, *iss*, *iutA*, *sitA*). In-ovo vaccination elicited enhanced mucosal immune responses, especially elevated IgA levels in cloacal swabs and lung tissue. Vaccinated chicks showed significant reduction in hepatic and ileal lesion scores and decreased prevalence of APEC in air sacs. Cytokine expression analysis revealed modulation of IL-1 β and IL-10, while vaccinated groups also showed a trend toward reduced bacterial loads in liver and spleen. Additionally, a lower frequency of virulence genes was observed in vaccinated animals, indicating a protective effect. These findings support the use of in-ovo administration of nanoparticle-based antigens as a feasible strategy to stimulate early immune protection against APEC in poultry.

Keywords: broiler pathology;in ovo vaccination;avian pathogenic *E. coli*.

2782 - Category: VACCINES (VC)

Production of anti-ES24 antibodies from hyperimmunized sheep for the control of *Haemonchus contortus*

KAPRITCHKOFF, R.T.I.¹; DE CAMPOS, E.M.¹; BELLO, H.J.S.²; DA CUNHA, A.F.³; DA COSTA, E.C.³; QUEIROZ, M.G.A.³; CORRÊA, L.G.P.⁴; ESTEVES, S.N.²; CHAGAS, A.C.D.S.²; OKINO, C.H.². 1. FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNIVERSIDADE ESTADUAL PAULISTA (UNESP), JABOTICABAL - SP - BRASIL; 2. EMBRAPA PECUÁRIA SUDESTE, SÃO CARLOS - SP - BRASIL; 3. CENTRO UNIVERSITÁRIO CENTRAL PAULISTA (UNICEP), SÃO CARLOS - SP - BRASIL; 4. FACULDADE DE CIÊNCIAS AGRÁRIAS E TECNOLÓGICAS - CÂMPUS DE DRACENA, DRACENA - SP - BRASIL.

Haemonchus contortus, the main gastrointestinal nematode parasitizing sheep, has developed resistance to all classes of anthelmintics, making it essential to study alternative control methods that reduce reliance on these drugs. This study aimed to develop a passive immunization protocol by producing specific antibodies against the 24 kDa excretory-secretory protein of *H. contortus* (ES24), which has been associated with immune protection against this parasite. The experiment was conducted at Embrapa Southeast Livestock (São Carlos, SP), using 10 Santa Inês sheep (G1), aged between 8 and 12 months, naturally infected with *H. contortus*. Each animal received a vaccine emulsion containing ES24 plus adjuvants, administered subcutaneously above the right scapula. Another group (G2), consisting of 6 sheep of the same breed, age, and kept in the same paddock, did not receive immunization. Weekly, blood samples were collected to evaluate packed cell volume (PCV), and fecal samples were collected to perform fecal egg counts (FEC) starting from D0 (day of first immunization). Serum samples were also collected at these intervals to measure anti-ES24 IgG, IgM, and IgA isotypes by indirect ELISA. On D14 and D28, additional serum samples were obtained using blood collection bags without anticoagulant, within the limit of 10 mL per kg of body weight, for large-scale production of specific antibodies. The results showed that G1 had higher levels of anti-ES24 antibodies for all isotypes compared to G2, with a notable increase in IgM on D14, which is associated with greater protection against *H. contortus*. During the same period, G1 also showed higher PCV values and lower FEC, indicating better infection control. Given the strong immune response observed in the immunized sheep, the protocol developed may become a viable and promising immunoprophylactic strategy to help control *H. contortus* in sheep.

Keywords: IgM;Indirect ELISA;Resistance.

2812 - Category: VACCINES (VC)

The first Brazilian mRNA Vaccine: Immune Response Insights Against SARS-CoV-2

JORGE, R.G.A.D.C.; TUBARÃO, L.N.; MACHADO, T.L.; MULLER, R.; SANTOS, R.T.; DA SILVA, J.F.A.; BOM, A.P.D.A.; NEVES, P.C.D.C.; MELGAÇO, J.G.. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

COVID-19 pandemic emphasized the urgent need for vaccines capable of eliciting effective and durable immune responses. Among the platforms available, messenger RNA vaccines have shown the ability to induce robust humoral and cellular immunity, with high antigen specificity and rapid adaptability to emerging variants. This study presents a preclinical immunological evaluation of the Brazilian mRNA vaccine prototype against SARS-CoV-2, focusing on antigen expression and the induction of specific immune responses. The objective was to assess the in vitro expression efficiency of the Spike antigen in HEK-293T cells, and to characterize, in vivo, the immune profile elicited by the vaccine, including IFN- γ production and anti-Spike IgG antibody titers. HEK-293T cells were transfected with the prototype, naked RNA, and a commercial mRNA vaccine to compare antigen expression. For the in vivo experiments, transgenic K18-hACE2 mice were immunized with either the prototype or the commercial vaccine. The humoral response was assessed via ELISA quantification of antigen-specific IgG antibodies, while the cellular response was analyzed through ELISPOT assays for IFN- γ secreting cells following stimulation with Spike-derived peptides. Results demonstrated that the prototype vaccine induced higher antigen expression in vitro compared to the commercial formulation. In vivo, mice vaccinated with the prototype displayed elevated levels of Spike-specific IgG antibodies and a significantly higher frequency of IFN- γ -producing T cells, indicative of a strong Th1-biased cellular immune response. The findings suggest that the Brazilian mRNA vaccine prototype induces a balanced and robust immune response, with significant activation of both adaptive humoral and cellular immunity, contributing to effective viral control. The ability to induce strong IFN- γ responses underscores its relevance in viral infections where cell-mediated immunity plays a crucial role in viral clearance and protection.

Keywords: RNAm vaccine; SARS-COV2; Immune response;.

2823 - Category: VACCINES (VC)

Effectiveness of Intranasal Administration of LaAg in Golden Hamsters (*Mesocricetus auratus*) Against Visceral Leishmaniasis

DE SÁ, B.D.S.¹; RIBEIRO, H.C.G.¹; DA-CRUZ, A.M.¹; ROSSI-BERGMANN, B.²; PINTO, E.F.¹; GUEDES, H.L.D.M.¹; MARTINS, A.M.D.F.¹. 1. INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis comprises a complex of parasitic diseases caused by protozoa of the genus *Leishmania*, encompassing 20 species pathogenic to humans. Transmission occurs through the blood meal of infected female sandflies. Clinical manifestations of leishmaniasis are diverse, including localized cutaneous, mucosal, diffuse leishmaniasis, and visceral leishmaniasis, the latter being fatal if untreated. Current treatment options are problematic due to high medication toxicity, and no vaccines are yet approved for human use. Therefore, developing a safe vaccine is crucial for leishmaniasis prevention. In this study, we utilized total antigens from whole promastigotes of *Leishmania amazonensis* (LaAg) administered intranasally as a vaccine candidate to assess protection against *L. infantum* infection, the causative agent of visceral leishmaniasis. Initially, we evaluated *L. infantum* infection in hamsters at different parasite doses. Over a 3-month period, we monitored animal weight and subsequently analyzed spleen, liver, and bone marrow tissues. Parasite load was quantified using a limiting dilution assay, revealing disease development across different parasite doses. Subsequently, we assessed the LaAg vaccine's efficacy under high *L. infantum* challenge conditions to determine protection against infection overload. Hamsters received two doses of LaAg (10 μ g/20 μ L) intranasally, spaced 15 days apart, followed by a high-dose *L. infantum* challenge. Preliminary findings indicated that under this high infection load, the vaccine did not confer protection in animals. Our future research will focus on evaluating vaccine effectiveness under lower infection doses.

Keywords: L; infantum; LaAg; vaccine.

2836 - Category: VACCINES (VC)

Immunological Potential of Engineered Extracellular Vesicles as Carriers of *Plasmodium vivax* Antigens

DA VEIGA, G.T.S.; DE LIMA, J.D.; ALBRECHT, L.. FIOCRUZ-PR, CURITIBA - PR - BRASIL.

INTRODUCTION: Malaria remains one of the most widespread parasitic diseases globally, posing serious challenges in endemic regions. Developing a safe and effective vaccine, especially against *Plasmodium vivax*, remains a major challenge. Current vaccine candidates exhibit limited immunogenicity. Extracellular vesicles (EVs) have emerged as promising antigen delivery platforms due to their natural ability to interact with immune cells. This study aimed to develop engineered EVs carrying the *P. vivax* gametocyte antigen Pvs47 and evaluate their interaction with immune cells.

METHODOLOGY: The gene encoding Pvs47 was integrated into the ExpiCHO cell genome. GFP-positive cells were enriched via flow cytometry. EVs were isolated from culture supernatants by differential centrifugation. Characterization included nanoparticle tracking, electron microscopy, and mass spectrometry. Fluorescently labeled EVs were used in uptake assays with monocytes. Immune activation was assessed by ICAM-1 expression, and cytotoxicity was evaluated in apoptosis assays, both by flow cytometry.

RESULTS: After three rounds of cell sorting, a stable GFP-positive ExpiCHO cell line (~94%) was established, producing approximately 2×10^{12} EVs/mL with an average diameter of 200 nm. Morphological analysis confirmed the presence of a lipid bilayer. Proteomic profiling identified the Pvs47 peptides and EV markers such as CD81 and revealed 319 differentially expressed proteins. Internalization assays showed that up to 45% of monocytes incorporated the EVs. Endothelial cells treated with EVs showed a modest (~10%) increase in ICAM-1 expression, in a dose-dependent manner. Importantly, no cytotoxic effects were observed following EV exposure.

CONCLUSION: ExpiCHO cells effectively produce EVs capable of delivering *P. vivax* antigens to immune cells. These EVs represent a promising platform for targeted antigen delivery may contribute to the development of vaccines against malaria and other infectious diseases.

Keywords: vaccine;malaria;delivery.

2838 - Category: VACCINES (VC)

Development and evaluation of recombinant antigen rLeish by intranasal route against experimental infection of *Leishmania amazonensis*

PEIXOTO, G.A.D.S.¹; RODRIGUES, N.C.²; GOMES, P.S.²; GUEDES, H.L.D.M.². 1. INSTITUTO OSWALDO CRUZ - IOC/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis is a worldwide public health problem caused by *Leishmania* spp. but doesn't have licensed vaccines for humans. Previous data showed that intranasal immunization of LaAg is capable to protect mice against *L. amazonensis*. Our group has been investigating new candidates, exploring not only conventional routes, but also mucosal vaccination. Herein, we aimed to produce a recombinant antigen (rLeish) and analyze their efficacy as an intranasal vaccine against *L. amazonensis*. rLeish was expressed at high levels and obtained at soluble and insoluble forms using pET28a vector. Besides that, we could recover great quantities of purified antigen using affinity chromatography, indicating an efficient production of rLeish. Interestingly, the immunogenicity of rLeish intranasal immunization was observed in mice that received only rLeish, leading to an increase in the footpad thickness after challenge. This indicates the capacity of rLeish to generate an immune response by intranasal route and to produce a response that recognizes antigens from the infection. Furthermore, the immunization with rLeish could protect against *L. amazonensis*. Mice vaccinated only with rLeish significantly control the lesion growth, but rLeish had its protection improved when administered with adjuvants, being able to further control the lesion size. However, the parasite burden was not impacted by rLeish, indicating that the mechanisms that control the lesion aren't the same as those that control the parasite load. Lymph nodes analysis has indicated that mice immunized with rLeish alone had a decrease of CD4+ FOXP3+ T cells, specifically in CTLA-4+. In the other hand, rLeish with adjuvant had an increase of CD8+ and IFN- γ -producing CD4+ T cells, but no difference in CD4+ FOXP3+, suggesting a different type of immune response. These data suggests that rLeish are a promising candidate for a vaccine and reinforces the mucosal vaccination as a relevant route against leishmaniasis.

Keywords: Vaccine;Leishmania;Intranasal.

2858 - Category: VACCINES (VC)

Experimental vaccine against leptospirosis: evaluation of the *Leptospira* metalloprotease leptolysin as a vaccine antigen

AMAMURA, T.A.¹; COURROL, D.D.S.²; BARBOSA, A.S.²; CHURA-CHAMBI, R.M.²; ISAAC, L.¹. 1. INTITUTE OF BIOMEDICAL SCIENCE, SÃO PAULO - SP - BRASIL; 2. BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL.

Leptospirosis is a zoonosis caused by spirochetes of the genus *Leptospira*, highly prevalent in tropical and subtropical regions, and represents a serious public health concern. Currently available vaccines exhibit significant limitations, including low efficacy, adverse effects, short-lived immunity, and lack of cross-protection among serovars. In previous studies, we demonstrated that recombinant leptolysin, a metalloprotease secreted by pathogenic *Leptospira*, interferes with macrophage-mediated bacterial phagocytosis by degrading opsonins such as C3b and iC3b, thereby promoting immune evasion. In this study, we evaluated recombinant leptolysin as a vaccine candidate. Golden Syrian hamsters and C3H/HeJ mice (Toll-like receptor 4-deficient) were immunized using aluminum hydroxide as adjuvant and subsequently challenged with *Leptospira interrogans* (strain L1-130). Experimental standardization identified 60 µg as the optimal leptolysin dose, while the 50% infectious doses (ID₅₀) were established as 1×10⁶ leptospires/mL for hamsters and 1×10⁸ leptospires/mL for mice. Immunization elicited high levels of anti-leptolysin antibodies in both models, confirming strong immunogenicity. Despite this robust humoral response, qPCR (Quantitative Polymerase Chain Reaction) analysis revealed the persistence of leptospires in the kidneys, indicating that immunization did not fully prevent renal colonization. Nonetheless, immunized mice maintained body weight after challenge, suggesting attenuation of clinical signs. Next steps include repeating the lethal challenge in 8-week-old hamsters, quantifying bacterial burden in liver and lungs, conducting histopathological analysis for inflammatory lesions and fibrosis, and performing immunohistochemistry to detect leptospires in tissues. These findings will provide further insight into the efficacy of leptolysin as a vaccine antigen and the immune mechanisms involved in protection against leptospirosis.

Keywords: leptolysin;leptospira;vaccine.

2865 - Category: VACCINES (VC)

Protection against a swine H1N1 and the highly virulent H5N1 (2.3.4.4b) heterosubtypic isolates induced by DNA -based vaccine encoding the influenza nucleoprotein

FAUSTINO, L.P.¹; SILVA, G.D.A.¹; DE SOUZA, N.S.H.²; FIGUEIREDO, M.M.¹; DE SOUZA, E.E.²; GAMON, T.H.³; WRENGER, C.³; DURIGON, E.³; TEIXEIRA, S.M.R.²; GAZZINELLI, R.T.². 1. CENTRO DE TECNOLOGIAS DE VACINAS - CT VACINAS, BELO HORIZONTE - MG - BRASIL; 2. INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO - USP, SÃO PAULO - SP - BRASIL.

Influenza virus hemagglutinins, the main target for neutralizing antibodies, constantly mutate, resulting in reduced vaccine efficacy across different seasons. In contrast, nucleoprotein (NP) is highly conserved, abundant in infected cells, and immunogenic. In this work, we developed a DNA vaccine for influenza that encodes the NP protein from A/PR/8/34 (H1N1). The sequence encoding NP was inserted into pCTV1, resulting in the pCTV1-NP. The expression of NP by pCTV1-NP in HEK cells was confirmed through immunofluorescence and Western blot analyses. Next, C57BL/6 mice were immunized intramuscularly. Significant levels of total IgG anti-NP were detected in the sera and bronchoalveolar lavage fluid of immunized mice. Furthermore, a robust cellular immune response was observed. Splenocytes from pCTV1-NP immunized animals produced IFNγ in response to restimulation with PR8 lysate or NP, compared to the control group that received the empty pCTV1. The pCTV1-NP formulation protected against H1N1 swine and highly pathogenic H5N1 influenza (2.3.4.4b clade). Immunized mice challenged and euthanized at 5 dpi maintained their body weight and survived the infection. Regarding viral load, lung samples from pCTV1-NP-immunized mice showed a reduction compared to those from pCTV1 controls. Analysis of cellular responses revealed that mice immunized with pCTV1-NP and challenged exhibited a strong IFNγ-producing CD8+ T cell response upon stimulation with NP-specific peptides or full-length NP. In contrast, splenocytes from control mice showed minimal responses when stimulated. Histopathological analyses showed no lung damage in immunized animals, whereas the lungs of control animals exhibited areas of parenchymal infiltration by inflammatory cells, perivascular infiltrates, and peribronchial infiltrates. Altogether, our results indicate that the plasmid DNA-NP vaccine encoding the NP protein could be used as an immediate frontline vaccine to confront epidemic and pandemic outbreaks.

Keywords: Influenza;DNA-based vaccine;Nucleoprotein.

2875 - Category: VACCINES (VC)

Developing chimeric vaccine antigens for sustainable control of *Rhipicephalus microplus*

FISCH, A.¹; DE MORAES, A.²; MENDES, N.S.²; LEITE, L.D.C.T.²; BARROS, P.H.A.³; MILANEZI, C.M.²; GARDINASSI, L.G.A.²; BRIGIDO, M.D.M.³; FERREIRA, B.R.². 1. THE ROSLIN INSTITUTE, EDINBURGH - REINO UNIDO; 2. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

Rhipicephalus microplus, the cattle tick, is considered a major One Health problem that negatively affects global livestock, highlighting the urgent need for sustainable control strategies. Our group previously developed a multi-antigen anti-tick vaccine with approximately 78% efficacy. However, its commercial feasibility is limited due to the need for multiple fermentations. To overcome this limitation, we are developing new anti-tick vaccines using two strategies: a) chimeric proteins that combine full sequences of three different protective antigens each (trivalent); and b) a multi-epitope single antigen composed of epitopes identified within ten protective antigens. Trivalent chimeric antigens were successfully produced in *E. coli* expression system, exhibiting purity and feasibility for scalable production, and are ready for the *in vivo* test. For the multi-epitope strategy, refined B- and T-cell epitope mapping was conducted across ten protective antigens. Sera from anti-tick protected bovines were screened using Peptide Microarray and Next-Generation Phage Display (NGPD). Six linear B-cell epitopes were identified in 4/9 antigens through Peptide Microarray, while NGPD revealed one to three conformational B-cell epitopes per antigen. Additionally, immunoinformatic analysis predicted 17 CD4 T-cell epitopes across all ten antigens, covering seven BoLA-DR alleles representative of South American herds. These epitopes were incorporated into the design of a chimeric, epitope-based antigen that was evaluated *in silico* and demonstrated favorable physicochemical and biological parameters for use in recombinant vaccines. Our results support the feasibility of developing scalable, cost-effective, and commercially viable chimeric vaccines for the control of *R. microplus* in cattle. Support: FAPESP- 23/04771-3; 22/07400-3.

Keywords: Anti-tick vaccine; *Rhipicephalus microplus*; Bovines.

2893 - Category: VACCINES (VC)

CELLULAR AND HUMORAL NATURALLY ACQUIRED IMMUNE RESPONSES TO PLASMODIUM FALCIPARUM CHIMERIC VACCINE CANDIDATE GMZ2.6C IN INDIVIDUALS EXPOSED TO MALARIA LIVING IN THE BRAZILIAN AMAZON

BAPTISTA, B.D.O.¹; SOARES, I.F.¹; DE OLIVEIRA, L.S.¹; DE SOUZA, H.A.D.S.¹; DE BARROS, J.P.¹; RICCIO, E.K.P.¹; MARTORANO, R.M.²; THEISEN, M.³; RODRIGUES-DA-SILVA, R.N.¹; LIMA JUNIOR, J.D.C.¹; TOTINO, P.R.R.¹; DANIEL-RIBEIRO, C.T.¹; RICCIO, L.R.P.¹. 1. INSTITUTO OSWALDO CRUZ, FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO ACRE, CRUZEIRO DO SUL - AC - BRASIL; 3. UNIVERSITY OF COPENHAGEN, COPENHAGEN - DINAMARCA.

The GMZ2.6c vaccine candidate is a multi-stage *Plasmodium falciparum* chimeric protein containing fragments of 48/45 Surface Protein (Pfs48/45), Glutamate-rich Protein (GLURP), and Merozoite Surface Protein-3 (MSP-3). This study aimed to evaluate the immune response against GMZ2.6c and its components, and to identify immunodominant B- and T-cell epitopes in individuals exposed to malaria in the Brazilian Amazon. Antibodies against GMZ2.6c, MSP-3, GLURP, Pfs48/45, and predicted B-cell epitopes were detected by Enzyme-Linked Immunosorbent Assay. Peripheral blood mononuclear cells (PBMCs) were stimulated with GMZ2.6c and its components, and central (CM)/effector (EM) memory T cells and activation markers were analyzed by flow cytometry. Cytokine concentrations were assessed by Cytometric Bead Array. Specific Interferon-gamma (IFN- γ)-secreting T-cells against predicted T-cell epitopes were determined by Enzyme-Linked Immunosorbent Spot. IgG antibodies against MSP-3 and GLURP were purified and used in immunofluorescence and opsonization assays. GMZ2.6c was widely recognized by antibodies. The higher prevalence of individuals with antibodies against GMZ2.6c compared to its components suggests an additive effect of GLURP, MSP-3, and Pfs48/45 when inserted in the same construct. Individuals had cellular immune responses against MSP-3 and Pfs48/45 capable of recalling T-cell responses upon GMZ2.6c stimulation. *P. falciparum* infection might modulate the immune response by reducing CD4⁺T_{EM} cells after GMZ2.6c and Pfs48/45 stimulation and inducing a prominent pro-inflammatory response. P11 (from GLURP), MSP-3c and DG210 (from MSP-3) were the immunodominant B-cell epitopes. Most individuals had IgG against Pfs48/45a and/or Pfs48/45b. All pools containing T-cell epitopes induced IFN- γ responses. Anti-MSP-3 and -GLURP antibodies recognized the native proteins and promoted a high opsonizing activity. Our data highlights the importance of GMZ2.6c as a multistage vaccine candidate.

Keywords: GMZ2.6c; Immune response; *Plasmodium falciparum*.

2906 - Category: VACCINES (VC)

Enhanced IgG Concentration and Avidity in Bovine Colostrum Following SARS-CoV-2 RBD Immunization with NIBDAF-Based Nanoadjuvants

CARVALHO, W.A.¹; BONATTO, C.C.²; GASPAR, E.B.¹; BASTOS, A.P.³; SOUZA, P.E.D.A.⁴; HONORIO, N.N.⁴; PRUDENCIO, C.R.⁵; DE GASPARI, E.⁵; DOMINGUES, R.¹; DIAVAO, J.¹; CAMPOS, M.M.¹; MARTINS, M.F.¹; MACHADO, M.A.¹; BRANDÃO, H.D.M.¹; SILVA, L.P.². 1. EMBRAPA GADO DE LEITE, JUIZ DE FORA - MG - BRASIL; 2. EMBRAPA CENARGEN, BRASILIA - DF - BRASIL; 3. EMBRAPA SUÍNOS E AVES, CONCÓRDIA - SC - BRASIL; 4. UFJF, JUIZ DE FORA - MG - BRASIL; 5. INSTITUTO ADOLFO LUTZ, SÃO PAULO - SP - BRASIL.

Hyperimmune bovine colostrum has emerged as a scalable and safe source of neutralizing antibodies for passive mucosal immunization. The quality and persistence of antibody responses are influenced by the choice of adjuvants. A phagocyte target delivery immunomodulatory nanosystem (NIBDAF; ongoing intellectual property protection) may potentiate both the magnitude and avidity of the antibody response. To compare total IgG concentration and avidity in serum and colostrum of cows immunized with SARS-CoV-2 recombinant RBD protein combined with different adjuvants: Quil-A®, Alúmen®, NIBDAF, and NIBDAF + Alúmen®. Holstein cows in late gestation received three intramuscular doses of recombinant RBD protein formulated with one of the adjuvants. Serum and colostrum samples were collected postpartum. Total IgG levels were measured by indirect ELISA, and avidity was evaluated using a 6 M urea-elution ELISA. Avidity index (AI) was calculated as the ratio of OD values with and without urea. Statistical analysis was performed by two-way ANOVA followed by Tukey and Sidak's multiple comparisons test ($p < 0.05$). All adjuvant formulations elicited detectable IgG responses in serum and colostrum. The NIBDAF + Alúmen® group showed the highest mean IgG concentrations in colostrum (89.5 ± 5.8) and serum (98.6 ± 6.5). Antibodies from all groups exhibited high avidity (AI > 65%). However, colostrum from cows immunized with NIBDAF + Alúmen® had significantly higher AI compared to Alúmen® alone ($p = 0.0425$), and serum AI was significantly higher than in Quil-A® and Alúmen® groups ($p < 0.01$). Immunization with recombinant RBD encapsulated in NIBDAF nanoadjuvant co-administered with Alúmen®, enhances both the quantity and quality of the antibody response in bovine secretions. These findings underscore the potential of NIBDAF-based adjuvants for generating high-avidity antibodies in livestock and support their use in future passive immunization strategies targeting zoonotic respiratory pathogens.

Keywords: Bovine Colostrum ; Nanoadjuvants; SARS-CoV-2.

2974 - Category: VACCINES (VC)

Establishment and Standardization of a Recombinant VSV Platform Pseudotyped with Mpox Virus Envelope Proteins for Vaccine Development and Immunogenicity Studies

PINTO, L.; RUSSO, E.M.D.S.. FACULTY OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO (FCFRP) - USP, RIBEIRÃO PRETO - SP - BRASIL.

Recombinant vesicular stomatitis virus (rVSV) has attracted considerable interest as a novel platform for virus entry studies and vaccine candidate development. Pseudotyping using heterologous envelope proteins enables this application. However, successful implementation requires the optimization and mastery of key steps, including plasmid amplification and purification, mammalian cell culture, viral rescue, and replication analysis, all of which are critical for the efficient generation of rVSV. The main goal of this study was to develop a robust set of protocols in the production of rVSV while standardizing its characterization. Plasmids containing the green fluorescent protein (GFP), as well as VSV structural genes were amplified in *E. coli* DH5 α and purified using a commercial kit with following the manufacturer's instructions. Quality of the plasmids were assessed by both gel electrophoresis and spectrophotometry. HEK-293T cells were cultured in OptiPRO SFM with either 5% or 10% FBS at 37 °C in 5% CO₂. Lipotransfection was utilized to generate rVSV through the co-delivery of the plasmids. GFP fluorescence and formation of plaques demonstrated viral recovery. Growth curves were reconstructed from plaque assays performed at 0, 24, and 48 hours post-infection. Cell growth looked excellent and there was quite a lot of recovery of virus from the culture with titers doubling from 24 to 48 hours. Additionally, the production of rVSV particles pseudotyped with the Mpox virus envelope proteins is currently underway in an effort to produce functional particles for future entry and immunogenicity studies. This method not only yields high-quality rVSV from a validated system, but also establishes a solid foundation for downstream pseudotyping applications and vaccine vector development involving envelope proteins from a wide range of emerging and re-emerging enveloped viruses.

Keywords: Vaccine vector; Immunogenicity; Mpox virus.

2986 - Category: VACCINES (VC)

COMPARATIVE ANALYSIS OF VIRULENCE BETWEEN BCG STRAINS ISOLATED FROM PATIENTS DIAGNOSED WITH BCGITIS AND VACCINE STRAINS

RANGEL, B.A.B.¹; ALMEIDA, F.M.¹; LARA, F.A.²; SUFFYS, P.N.²; LASSOUNSKAIA, E.¹. 1. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE, CAMPOS DOS GOYTACAZES - RJ - BRASIL; 2. INSTITUTO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

The *Bacillus Calmette-Guérin* (BCG) vaccine remains the only approved vaccine for tuberculosis immunoprophylaxis in newborns. In most cases, it induces an immune response strong enough to eliminate the bacteria. Although complications are rare, susceptible or immunosuppressed individuals may develop adverse reactions, such as BCGitis, characterized by inflammation at the inoculation site and regional lymph nodes, or BCGosis, a systemic form of the disease. Increased residual virulence in some vaccine strains may contribute to these outcomes. Moreover, uncontrolled bacterial replication may favor the emergence of more competitive and virulent mutants. To explore this, a mouse model was used to compare the virulence of clinical BCG isolates (from localized or systemic infections) with their original vaccine strains. Isolates from patients in Argentina and Venezuela were inoculated intratracheally into C57BL/6 mice. After 14 and 28 days, bacterial survival, lung histopathology, and inflammation were assessed. Results showed that most clinical isolates, especially from disseminated cases, were more virulent than their respective vaccine strains. Although the immune system reduced bacterial load, the more virulent strains persisted longer, caused more intense and progressive lung granulomas, and spread more to the spleen. The investigation of genetic polymorphisms potentially associated with increased virulence is ongoing through whole-genome sequencing of clinical isolates and vaccine strains. These findings underscore the potential for increased virulence in bacteria that persist for extended periods in vaccinated individuals and highlight the importance of initiating antimycobacterial therapy promptly in cases of adverse reactions, as well as the need to improve future generations of the BCG vaccine.

Keywords: BCG;CLINICAL ISOLATES;VACCINE STRAINS.

3003 - Category: VACCINES (VC)

In silico design of a chimeric multi-epitope vaccine targeting conserved entry and exit proteins across the Orthopoxvirus genus

DE ARAÚJO, L.P.¹; SILVA, E.N.¹; AUGUSTO, L.V.¹; DE ALENCAR, S.M.²; CORSETTI, P.P.¹; DE ALMEIDA, L.A.¹. 1. UNIFAL-MG, ALFENAS - MG - BRASIL; 2. USP, PIRACICABA - SP - BRASIL.

The *Orthopoxvirus* (OPXV) genus includes several emerging and re-emerging zoonotic viruses such as Mpox and the recently described *Borealpox* virus, which caused the first human fatality in Alaska (2024). In response to rising outbreaks, the World Health Organization declared Mpox a public health emergency of international concern in both 2022 and 2024. Although two vaccines are currently available (ACAM2000 and JYNNEOS), both present notable limitations, particularly concerning immunogenicity and safety in immunocompromised individuals. Thus, the aim of this study was to identify conserved epitopes in viral proteins involved in the processes of viral entry and exit processes of host cells, encompassing all 16 known OPXV species, and to design a chimeric multi-epitope immunogenic vaccine using *in silico* methods. For this purpose, 160 proteins sequences were retrieved from the NCBI database, from all known OPXV viruses were analyzed to identify conserved epitopes using the Immune Epitope Database (IEDB), yielding 5,048 predicted T and B epitopes. These were filtered based on antigenicity, allergenicity, toxicity, transmembrane domains, and glycosylation sites. Eight highly conserved epitopes from 8 different proteins were selected. These were fused with PADRE and β -defensin adjuvants to construct a chimeric protein, which was structurally modeled using AlphaFold3. Structural validation included molecular docking with TLR2 and 100 ns molecular dynamics simulations using GROMACS. Immune response simulations (C-ImmSim) predicted robust IgM and IgG responses and high levels of IFN- γ , IL-10, and TGF- β following a three-dose schedule. This is the first study to report a multi-epitope vaccine design based on epitopes conserved across the entire OPXV genus. The results support its potential as a safe, immunogenic, and broadly protective vaccine candidate. Moreover, the identified epitopes may also serve as valuable targets for the development of rapid immunodiagnostic tools.

Keywords: Orthopoxvirus;Reverse vaccinology;Immunoinformatics.

3011 - Category: VACCINES (VC)

Immunogenicity of meningococcal OMVs formulated with alum and cholera toxin subunit B in mice at different developmental stages

SEGATI, A.F.; DE MELO, N.S.; DE GASPARI, E.. ADOLFO LUTZ INSTITUTE/UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL.

Young children and young adults are major risk groups for meningococcal disease; however, developing effective and safe vaccines for these populations remains challenging. In the Brazilian Unified Health System (SUS), vaccine coverage is insufficient for several serogroups, and the high variability of circulating strains compromises the efficacy of available vaccines. This study evaluated the immunogenicity of *Neisseria meningitidis* serogroup C outer membrane vesicles (OMVs) formulated with the adjuvants cholera toxin subunit B (CTB) and aluminum hydroxide (AH). BALB/c mice were immunized subcutaneously with three doses on days 10, 40, and 55 of life. The immune response was assessed by ELISA for total IgG and the isotypes IgG1, IgG2a, IgG2b, and IgG3, as well as IgG avidity. Immunoblotting identified OMV antigens recognized by IgG in the homologous strain, while Dot-Blot evaluated cross-reactivity with heterologous *N. meningitidis* strains. Significant IgG production was observed after the second dose ($p < 0.001$), increasing further after the third ($p < 0.0001$). All isotypes were detected, with IgG1 predominance, indicating a Th2-skewed response. Avidity was high after the second dose (60.31 ± 16.31) and increased after the third (76.35 ± 14.74), suggesting the induction of high-affinity antibodies. The third dose also enhanced the diversity and specificity of IgG antibodies that recognized antigens between 25–58 kDa and 100–190 kDa. Immune sera reacted with heterologous strains from serogroups C, W, and B, particularly serogroup B, prevalent in Brazil. The OMV+CTB+AH formulation showed strong potential to induce a robust and functional immune response, supporting its use in developing broader-coverage vaccines targeting the antigenic diversity of circulating *N. meningitidis* strains.

Keywords: *Neisseria meningitidis*;Outer membrane vesicles;Vaccines adjuvants.

3038 - Category: VACCINES (VC)

Vaccinal strategy for Herpes simplex virus type 1 and 2: VLP vaccine using HSV-1-derived amplicon vectors

GALANO, J.D.¹; MELENDEZ, M.E.²; SATO, M.N.³. 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL; 3. FACULDADE DE MEDICINA DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Herpes simplex type 1 (HSV-1) and type 2 (HSV-2) have co-evolved with humans and are present at high frequency in the general population, affecting approximately 190 million people in the world. The virus can cause serious neonatal infection, resulting in the development of encephalitis and keratitis. There are about 14.000 annual cases of neonatal infection in the world. Despite the low incidence, it can be associated with significant morbidity and mortality, especially when there is dissemination or involvement of nervous central system. HSV-1 recombinant vectors have a large transgene capacity (~150 kb), broad cell tropism, low immunogenicity and toxicity and easy manipulation, the use of this platform has showed potential results as vaccines for West Nile virus, Foot-and-mouth disease virus and rotavirus. The purpose of this project is to produce a vaccine, using a system of HSV-1 recombinant vectors to express the glycoprotein C of HSV-2 (gC2) and to evaluate its response in female Balb/c mice (CEUA n° 2040/2024). gC2 is involved in the initial binding of the virus to the host cell and can bind to the C3b protein of the complement. We amplified the DNA sequence that codifies gC2, using conventional PCR, cloned the fragment in the plasmid amplicon (pA_EAU2) and started the development of HSV-1 amplicon vector, following the helper virus LaLΔJ protocol. Also, we tested two different doses (10^6 and 10^7 PFU) of HSV-2 wild-type virus (HG52 strain) in mice via intravaginal infection and observed the development of genital inflammation and lesion. Until now there isn't a preventive vaccine for HSV-1 and HSV-2, so this would be an important action for public health against congenital/neonatal infections.

Keywords: Herpes simplex virus;congenital infection;HSV-1 amplicon vector vaccine.

3042 - Category: VACCINES (VC)

Meningococcal vesicles and SARS-CoV-2 protein: adjuvant effect, inner immunogenicity, and cross-reactivity potential

PORTILHO, A.I.¹; DA COSTA, H.H.M.¹; SANTOS, R.A.²; DUARTE-BARBOSA, M.³; CHAVES, A.P.C.⁴; PRUDENCIO, C.R.¹; JANINI, L.M.R.²; DE GASPARI, E.¹. 1. IMMUNOLOGY CENTER, ADOLFO LUTZ INSTITUTE/ POST-GRADUATE PROGRAM INTERUNITS IN BIOTECHNOLOGY, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY, PAULISTA SCHOOL OF MEDICINE, FEDERAL UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. DEPARTMENT OF MEDICINE, FEDERAL UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. DEPARTMENT OF MEDICINE, PAULISTA SCHOOL OF MEDICINE, FEDERAL UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL.

SARS-CoV-2 continues to pose global health challenges due to emerging variants and the need for broadly protective vaccines. *Neisseria meningitidis* (Nm) release outer membrane vesicles (OMV), suitable for immunization as antigen and adjuvant. This study aimed to evaluate whether OMV from Nm C:2a:P1.5 strain could enhance the immunogenicity of the receptor binding domain (RBD) of SARS-CoV-2 and induce cross-reactive responses. Neonate to young BALB/c (H2^d) mice received 3 doses of 1.5µg RBD (Wuhan)+0.5µg OMV (Nm C:2a:P1.5)+0.1mM Alum (AH). Controls received only RBD, or OMV+AH, or were naïve. Immune response was evaluated by ELISA, avidity-ELISA, ELISpot, and a Cytopathic effect-based virus neutralization test for SARS-CoV-2-Wuhan. Cross-reactive IgG was detected for RBD-Omicron BA.2, and pathogenic Nm isolated from Brazilian clinical cases: 368 (Nm B:4:P1.9), and 6951 (Nm W:2a:P1.5,2) strains. The RBD+OMV+AH group showed significantly higher IgG-RBD titers than the RBD (p<0.01) and naïve (p<0.001) groups, along with increased antibody avidity (71 ± 15% vs 24 ± 10%) and neutralizing titers (1:320 vs 1:40) compared to RBD alone. OMV-specific responses in the RBD+OMV+AH group were comparable to the OMV+AH group. IgG1/IgG2a ratios suggested a Th2-skewed response for RBD and a mixed profile for OMV. IFN-γ and IL-4 secretion were detected from splenocytes after stimulation with RBD or OMV. Cross-reactive IgG of intermediary avidity (44±16%) was detected against RBD-Omicron BA.2 in the RBD+OMV+AH group. This group also presented IgG binding to Nm 368 with high avidity (78±6), and to Nm 6951 with intermediary avidity (49±16). These findings suggest that OMV-based adjuvant enhances SARS-CoV-2 RBD-specific immunity while preserving a meningococcal response, and supports broad cross-reactivity to antigens relevant to the Brazilian epidemiological context, indicating a potential for multivalent vaccine strategies.

Keywords: Outer membrane vesicles;COVID-19;Neutralizing antibodies.

3061 - Category: VACCINES (VC)

Combination of polysaccharides and outer membrane vesicles of *Neisseria meningitidis* induces IgG antibodies with high avidity

DE MELO, N.S.; PORTILHO, A.I.; SEGATI, A.F.; DE GASPARI, E.. UNIVERSIDADE DE SÃO PAULO, INSTITUTO ADOLFO LUTZ, SÃO PAULO - SP - BRASIL.

Neisseria meningitidis causes meningococcal disease, a public health concern, which relies on adequate immunization for its control. Current meningococcal vaccines, such as the pentavalent MenABCWY, offer broad protection but are technologically complex and costly. This study evaluates a simplified and potentially effective formulation using outer membrane vesicles (OMV) from the C:2a:P1.5 strain, combined with polysaccharides A (PSA) and C (PSC), to increase antigenic diversity. Isogenic A/Sn (H2a) adult mice were immunized intramuscularly with three doses of preparations containing 5µg of OMV, 2.5µg of PSA/PSC, and 0.1 mM/ml of alum (AH), the control group received only AH. Antibody response was evaluated through IgG, IgG1, IgG2a IgG2b, IgG3 levels. Preliminary results showed high IgG titres in OMV+PSC+AH (1st: 6,20 ± 0,32; 2nd: 7,64 ± 0,76; 3rd: 7,57 ± 0,41), OMV+PSA+AH (1st: 5,68 ± 0,26; 2nd:7,30 ± 0,36; 3rd: 7,28 ± 0,49) , and OMV+PSA+PSC+AH (1st:5,77 ± 0,59; 2nd: 7,30 ± 0,35; 3rd: 7,56 ± 0,31) groups, reaching 5 to 8 fold increases. Statistical difference was observed on these experimental groups compared to control (p<0,001). A mixed Th1/Th2 response was suggested by the IgG1/IgG2a ratio, after the first dose in OMV+PSC+AH (1,09 ± 0,54), OMV+PSA+AH (1,188 ± 0,77), and OMV+PSA+PSC+AH (1,73 ± 1,29), indicated by the balanced levels between IgG1 and IgG2a, shifting to a Th1 predominance in subsequent doses in OMV+PSC+AH (2nd: 0,3139 ± 0,09, 3rd: 0,21 ± 0,03), OMV+PSA+AH (2nd: 0,32 ± 0,09 3rd: 0,21 ± 0,03) and OMV+PSA+PSC+AH (2nd: 0,33 ± 0,15, 3rd: 0,24 ± 0,05). Notably, IgG2b and IgG3 were detectable only after the 2nd and 3rd doses, also at 6 to 10-fold higher increases. Avidity assay indicates the binding strength between the antibody and the antigen. Here, high avidity was detected across all groups. These results indicate a good antigenic formulation when meningococcal OMV are associated with PSA or PSC and AH.

Keywords: Outer membrane vesicles;Neisseria meningitidis;vaccines.

3095 - Category: VACCINES (VC)

Immune response to *Corynebacterium diphtheriae*-adhesin DIP0733, a potential alternative vaccine candidate against diphtheria

DO NASCIMENTO, I.V.¹; PEREIRA, B.C.D.S.¹; SILVA, M.V.D.M.¹; DA SILVA, M.T.L.¹; BULLA, A.C.S.²; ALLONSO, D.¹; LEAL, M.¹; OLSEN, P.C.¹; MOREIRA, L.O.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Corynebacterium diphtheriae is the main etiological agent of diphtheria, a severe upper respiratory tract infection. Despite the reduction of cases due to toxoid vaccination, the disease remains endemic worldwide, with higher case numbers in Africa and Southeast Asia countries. Additionally, a significant increase in non-toxinogenic isolates, associated with invasive diseases, and the detection of toxin mutations has been reported. In this context, studying other immunogenic virulence factors associated with bacterial pathogenesis, beyond diphtheria toxin, is urgent for developing an alternative or complementary vaccine candidate. Here, we investigated the immunogenicity of the DIP0733 surface adhesin protein from *C. diphtheriae*. For that, the recombinant protein was expressed and purified from *E. coli*. The r-DIP0733 protein was conformationally stable as shown by the circular dichroism and intrinsic tryptophan fluorescence assays. Bioinformatics analysis revealed that r-DIP0733 presents 19 potential epitopes for human antibodies. In vitro analysis showed that r-DIP0733 was able to induce nitric oxide and TNF- α by RAW macrophages. In vivo studies showed that r-DIP0733 immunization induced a robust production of IgM and IgG antibodies in mice, and IgG1, IgG2c, and IgG2b subclasses were detected. Furthermore, immunization with the heat-inactivated *C. diphtheriae* generated IgM and IgG antibodies against the r-DIP0733. In conclusion, r-DIP0733 presents a promising alternative for the development of new diphtheria vaccine that could be combined with diphtheria toxoid to increase diphtheria prevention.

Keywords: VACCINES;DIPHThERIA;DIP0733.

3096 - Category: VACCINES (VC)

Cow Immunization with Recombinant SARS-CoV-2 RBD Induces High-Avidity IgG in Bovine Colostrum and Transitional Milk

SOUZA, P.E.D.A.¹; PEIXOTO, L.L.T.D.²; GASPAR, E.B.³; BASTOS, A.P.⁴; PRUDENCIO, C.R.⁵; DOMINGUES, R.³; DE GASPARI, E.⁵; MARTINS, M.F.³; DIAVAO, J.³; CAMPOS, M.M.³; CARVALHO, W.A.³. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. CENTRO UNIVERSITÁRIO PRESIDENTE ANTÔNIO CARLOS, JUIZ DE FORA - MG - BRASIL; 3. EMBRAPA GADO DE LEITE, JUIZ DE FORA - MG - BRASIL; 4. EMBRAPA SUÍNOS E AVES, CONCÓRDIA - SC - BRASIL; 5. INSTITUTO ADOLFO LUTZ, SÃO PAULO - SP - BRASIL.

COVID-19 continues to be a global health concern beyond the pandemic phase. Bovine colostrum, naturally enriched in immunoglobulins, particularly IgG, offers a promising platform for passive immunization against SARS-CoV-2. Immunization of cows in the final third of gestation can induce neutralizing antibodies secreted in both colostrum and transition milk which is produced between days 2 and 10 postpartum. The aim of this work was evaluate the avidity of anti-SARS-CoV-2 IgG in serum, colostrum and transition milk from Holstein cows immunized with recombinant RBD protein formulated with Alum-Inject® or Quil-A® adjuvants. Serum, colostrum and milk samples (days 3, 5, and 10 postpartum) were collected. Antibody avidity was measured by urea elution ELISA (6 M), and the avidity index (AI) was calculated as the OD ratio ($\pm$ urea). For this purpose, a modification of the ELISA assay for quantifying total IgG in milk, colostrum and serum, previously standardized in the laboratory with anti-bovine IgG antibodies (Bethyl), was performed. Statistical comparisons were made using the Tukey and Sidak tests ($P < 0.05$). Both adjuvants induced high-avidity anti-RBD IgG in colostrum and milk (AI > 65%), with no significant difference between groups. Immunization with recombinant RBD protein effectively induces high-avidity antibodies in serum, bovine colostrum and transition milk, with no distinction between the adjuvants. These findings reinforce the potential of hyperimmune dairy products as a source of functional immunoglobulins. Further studies are needed to validate their protective efficacy as a nutraceutical in animal disease models.

Keywords: Avidity antibodies;Hyperimmune colostrum;SARS-CoV-2.

3131 - Category: VACCINES (VC)

A Systems Vaccinology Study Between Humans and Animal Models for Translational Medicine

SYED, W.A.P.¹; DE MIRANDA, G.C.²; HAGAN, T.³. 1. INSTITUTO DE CIENCIAS BIOMEDICAS, SÃO PAULO - SP - BRASIL; 2. INSTITUTO DE MEDICINA TROPICAL, SÃO PAULO - SP - BRASIL; 3. CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER, CINCINNATI - ESTADOS UNIDOS DA AMERICA.

Vaccines have revolutionized modern medicine by protecting individuals through immune responses. However, differences in immune mechanisms across species, particularly between humans and animal models, pose challenges in translating preclinical findings to human applications. Using RNAseq and microarray data from different studies, and the COVID-19 Vaccination Atlas and 13 Vaccines Atlas that our groups have previously developed, we aim to identify conserved and species-specific immune pathways through differential expression analysis and machine learning modeling to translate interspecies immune responses. We expect to uncover biomarkers for vaccine efficacy, highlight the translational limitations of mouse models, and inform the design of next-generation vaccines. Results will be integrated into an interactive platform for comparative analysis, offering insights into immune mechanisms in vaccination and guiding vaccine development for diverse populations.

Keywords: animal models;vaccines;translational medicine.

3140 - Category: VACCINES (VC)

Microneedles for allergen-specific immunotherapy: from design to dendritic cell activation

ULLOA, J.E.¹; FLATOW, E.A.²; ANDRADE, L.M.³; BOSCARDIN, S.B.⁴; ALVES, W.A.⁵; RUSSO, M.⁴; KALIL, J.²; DE OLIVEIRA, V.L.⁶. 1. INSTITUTO DE CIÊNCIAS BIOMEDICAS, UNIVERSIDADE DE SÃO PAULO & MICRONEEDS BIOTECNOLOGIA, SÃO PAULO - SP - BRASIL; 2. INCOR – INSTITUTO DO CORAÇÃO DO HCFMUSP, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS & MICRONEEDS BIOTECNOLOGIA, BELO HORIZONTE - MG - BRASIL; 4. INSTITUTO DE CIÊNCIAS BIOMEDICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 5. UNIVERSIDADE FEDERAL DO ABC, SANTO ANDRÉ - SP - BRASIL; 6. INCOR – INSTITUTO DO CORAÇÃO DO HCFMUSP & MICRONEEDS BIOTECNOLOGIA, SÃO PAULO - SP - BRASIL.

Microneedles (MNs) are ideal for skin delivery, making them highly relevant for transdermal immunotherapy and immunomodulation. Skin-resident dendritic cells (DCs) play a central role by presenting antigens and co-stimulatory signals to T cells. This interaction promotes T cell activation, differentiation, and proliferation, leading to a targeted immune response. Our main objective is to develop and validate a transdermal device based on MNs for allergen-specific immunotherapy.

Polycaprolactone (PCL) MNs were fabricated and coated with ovalbumin (OVA). Structural and dimensional characterization was performed using scanning electron microscopy (SEM). Mechanical strength and insertion efficiency of PCL MNs were evaluated via force–displacement analysis, piercing tests, optical coherence tomography (OCT), and transepidermal water loss (TEWL) measurements. Confocal microscopy was used to assess spatial distribution and release profile of OVA-Alexa Fluor™ 488 from MNs. Cytotoxicity of PCL MNs was tested *in vitro* using HaCaT human keratinocyte cell line. Allergen content and stability after five months at room temperature were measured by ELISA and Western blot. Efficient delivery of fluorescent OVA to monocyte-derived DCs and their activation were demonstrated by flow cytometry.

In summary, we developed and characterized a MN-based platform for allergen-specific immunotherapy, demonstrating its mechanical reliability, biocompatibility, and immunomodulatory potential. Further *in vivo* studies and biological safety tests are required to support regulatory submission and approval of this technology as a medical device for human use.

Footnote: Fapesp #2022/14753-0, INCT-CNPQ-iii #408685/2024-7

Keywords: Microneedle delivery;allergen-specific immunotherapy;Biomaterials.

3154 - Category: VACCINES (VC)

RNA vaccines platform implementation in FIOCRUZ/Brazil with an innovative tuberculosis vaccine development

MELGAÇO, J.G.¹; JORGE, R.G.A.C.¹; PEREIRA, T.B.S.¹; ROSA, L.C.¹; JÚNIOR, H.C.D.S.¹; DE SOUZA, R.S.¹; FREDERICO, A.B.T.¹; MACHADO, T.L.¹; TUBARÃO, L.N.¹; SPEROTTO, M.G.²; ANDRADE, B.D.B.³; CRODA, J.H.R.⁴; BOM, A.P.D.A.¹; NEVES, P.C.D.C.¹. 1. BIO-MANGUINHOS/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO MATO GROSSO DO SUL, CAMPO GRANDE - MS - BRASIL; 3. FIOCRUZ/BA, SALVADOR - BA - BRASIL; 4. FIOCRUZ/MS, CAMPO GRANDE - MS - BRASIL.

Tuberculosis (TB) remains a major global health issue, with Brazil reporting ~80,000 new cases in 2022. The BCG vaccine has limited efficacy in adults and declining coverage, reinforcing the need for new vaccination strategies. Messenger RNA (mRNA) vaccines offer a promising alternative, enabling host cells to produce specific antigens and trigger targeted immune responses. For TB, this approach could induce strong cell-mediated immunity against *Mycobacterium tuberculosis*.

Bio-Manguinhos, a WHO-selected center for RNA technologies, is developing an mRNA-based TB vaccine. Leveraging its flexible “plug-and-play” mRNA platform and lipid nanoparticle (LNP) formulations (validated in COVID-19 vaccines), preclinical studies assessed three native *M. tuberculosis* antigens through *in silico*, *in vitro*, and pilot *in vivo* assays.

In silico analyses showed strong activation of innate immunity through pattern recognition receptors and predicted B and T cell epitopes (rank <0.06). T cell epitopes indicated IFN- γ production potential by CD4⁺ Th1 cells (positive SVM score). *In vitro*, THP-1 monocytes transfected with the mRNA constructs showed enhanced M1 differentiation (%CD11b+CD11a+CD38⁺: control = 11.6, protein A = 44.2, B = 53.4, C = 42.5), supporting their capacity to act as antigen-presenting cells. *In vivo*, no clinical signs were observed in immunized mice, and flow cytometry revealed increased CD4⁺ Th1 activation in spleens first week post-immunization (p<0.05).

These findings demonstrate the feasibility of mRNA-based TB vaccines. Although still in early stages, the results are promising and support continued development to refine the strategy and ensure safety and efficacy.

Keywords: mRNA vaccine;Tuberculosis;prevention

3159 - Category: VACCINES (VC)

DEVELOPMENT OF A CANCER/TESTIS ANTIGENS mRNA VACCINE FOR THE TREATMENT OF HEAD AND NECK CANCER.

DO NASCIMENTO, A.L.C.¹; BOM, A.P.D.A.²; NEVES, P.C.D.C.². 1. FIOCRUZ/UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Cancer in the head and neck region, specifically in the oral cavity and pharynx, is predominantly caused by squamous cell carcinomas (SCCs), which account for over 90% of malignant neoplasms in these areas. Cancer-testis antigens (CTAs) are molecules typically expressed only in germ cells but reappear in cancer cells. Therefore, given the significant impact of head and neck cancer in Brazil and worldwide, the development of new therapies is essential. Objective: This project proposes the creation of a messenger RNA (mRNA)-based vaccine aimed at treating tumors in the oral cavity and pharynx. Methodology: The methodology involves the production of plasmid DNA through the transformation of *Escherichia coli*, followed by extraction using alkaline lysis. The obtained plasmid will be linearized for mRNA production via *in vitro* transcription. The mRNA will then be encapsulated in lipid nanoparticles (LNPs) using microfluidic techniques. LNP evaluation will include measuring size, polydispersity index (PDI), and zeta potential using dynamic light scattering (DLS), as well as verifying encapsulation efficiency. The effectiveness of antigen expression will be verified in transfected HEK-293T cells, with subsequent flow cytometry and western blotting assays to analyze antigen expression. Conclusion: Thus, it is expected that this vaccine will activate the immune system, leading to a decrease in tumor growth and an increase in survival in animal models. This proposal presents a promising alternative in the treatment of head and neck cancer.

Keywords: Cancer;Immunotherapy, mRNA;Nanotechnology.

3164 - Category: VACCINES (VC)

Evaluation of the protective capacity of recombinant *Leishmania* antigens RP2 α and RP3 β against *L.amazonensis* cutaneous infection model.

DA SILVA, V.A.C.¹; DO NASCIMENTO, L.A.²; RODRIGUES, N.C.²; MARTINS, A.M.D.F.²; GUEDES, H.L.D.M.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ) & FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis is a neglected parasitic disease with toxic, long and expensive treatment and no vaccines approved for human use to this day. Previous research on antileishmaniasis vaccines used classical immunization pathways, but some articles have highlighted the mucosal intranasal (IN) route protective capacity for novel antileishmaniasis vaccines. Research have shown that certain *Leishmania* proteins, such as serine-proteases and cysteine-proteases, has aggravating potential in LaAg vaccines. Recombinant technology is interesting for specific antigen production for underdevelopment countries, such as Brazil. Therefore, this work aimed to evaluate the protective capacity of RP2 α and RP3 β *Leishmania* spp. antigens, produced in *E.coli* BL21 (DE3), associated or not with Toll-Like Receptor Agonist (TLRA) or Oil-Water Emulsion (OWE) adjuvants, in BALB/c mice. Mice received 2 doses of RP3 β or RP2 α via IN route, with a 1-week gap between doses, associated or not with TLRA or OWE. 2 weeks after last immunization, mice received 2x10⁵ promastigotes of *L.amazonensis* (JOSEFA). Lesion size was evaluated per week. 59 days post infection, mice were euthanized and infected paw, lymph nodes and spleen were collected for parasite load evaluation using the limited dilution assay (LDA). In vivo experiment was conducted under the L-024/2024 CEUA license in a double-blinded standard. Results showed a reduction on lesion development in RP3 β +TLRA immunized mice with parasite burden reduction in the footpad, although no reduction was observed systemically (spleen and lymph nodes). A new *in vivo* experiment is ongoing, using RP3 β associated or not with 5 μ g or 15 μ g TLRA. In conclusion, we demonstrated the production of 2 soluble *Leishmania* spp. proteins in *E.coli* bacteria. When immunized, we showed partial protection against cutaneous *L.amazonensis* model in BALB/c mice immunized with RP3 β +TLRA, highlighting the potential of such formulation in a novel antileishmaniasis vaccine development.

Keywords: Recombinant Protein;Vaccine;Antileishmaniasis.

3211 - Category: VACCINES (VC)

Temporal Sleep Restriction Disrupts Vaccine-Induced Antibody and T Cell Responses in a Murine Model

PIRES, A.D.S.; NUNES, J.P.D.S.; KELLER, A.C.; TUFIK, S.; ANDERSEN, M.L.; ROSA, D.S.. UNIVERSIDADE FEDERAL DE SÃO PAULO - UNIFESP/EPM, SÃO PAULO - SP - BRASIL.

Sleep is a cyclical physiological state essential for maintaining homeostasis and supporting proper biological functioning. Increasing evidence demonstrates that sleep influences multiple organ systems and is associated with heightened disease susceptibility and poorer prognostic outcomes. There is a bidirectional relationship between sleep and the immune system, but the mechanisms by which sleep, and its alterations may influence immune responses are not yet fully understood. While studies in humans have shown that sleep restriction (SR) and sleep deprivation (SD) can reduce specific antibody titers following vaccination, data from animal studies investigating the impact of SD or SR on immune responses post-immunization are limited. In this study, we investigated the effects of SR on both humoral and cellular immune responses following immunization. Male C57BL/6 mice underwent SR at different time points: prior to immunization, immediately post-immunization, or during the period when antibody titers typically increase (5-9 days after 1st dose and 2-6 days after 2nd dose). Mice were immunized with two doses of ovalbumin (OVA) combined with the adjuvants AddaS03 and CpG-ODN. OVA-specific antibody production was measured by ELISA, while cellular immune responses were evaluated using T cell ELISpot and flow cytometry. The group subjected to SR during the period when antibody titers typically increase (5-9 days after 1st dose and 2-6 days after 2nd dose) exhibited reduced total OVA-specific IgG and IgG1 titers compared to healthy sleep group. Furthermore, all SR groups presented a reduced number of specific IFN γ -producing CD4⁺ T cells in the spleen and a lower frequency of follicular helper T cells (Tfh) in draining lymph nodes compared to healthy sleep group. These findings indicate that sleep restriction at critical time points can significantly impair both humoral and cellular immune responses to vaccination.

Keywords: Sleep Restriction;Vaccines;Immunity.

3218 - Category: VACCINES (VC)

Overexpression of ACE2 Receptor in VERO E6 Cells: A Model for SARS-CoV-2 Pseudovirus Infection and Neutralization Assays

REINHARDT, G.M.¹; PORTES JUNIOR, J.A.²; CALABRIA, P.A.L.²; GIMENES, S.N.C.²; MAGALHÃES, G.S.²; MAURO, E.F.D.L.². 1. INSTITUTO DE CIÊNCIAS BIOMÉDICAS, SÃO PAULO - SP - BRASIL; 2. BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL.

Neutralizing antibodies (NAbs) play a key role in eliminating viruses during infection, and their evaluation indicates protective immunity against reinfection. Cellular pseudovirus infection assays, such as SARS-CoV-2 (PVSARS) carrying the Luciferase gene, have been used to evaluate NABs and these assays require cell lines with high infection rates to avoid false negatives. The Immunopathology Lab-Instituto Butantan has been using the A549-hACE2 (A549+) cell line for this purpose. However, maintaining A549+ cell cultures requires specific procedures that raise *in vitro* assay costs. Thus, we proposed the use of the VERO E6 cell line, known for higher viral susceptibility. Preliminary results showed that VERO E6 was promising for PVSARS infection assays in our lab, though the luminescence values were lower than A549+. The project aimed to improve VERO E6 infectivity by overexpressing ACE2, the viral entry receptor, using VSV-G pseudovirus carrying the ACE2 (PVACE2). Initially, the pHACE2 plasmid was amplified and used to produce the PVACE2 in HEK293T cells. VERO E6 were infected with PVACE2, or with PVGFP as control. ACE2 overexpression was evaluated by: PVSARS infection; RT-qPCR and flow cytometry. Infection assays with PVSARS showed no significant differences between VERO E6+ and VERO E6. RT-qPCR showed a significant increase in ACE2 mRNA in VERO E6+, but flow cytometry did not confirm overexpression. Transient overexpression was hypothesized. A test in the same plate of PVSARS infection on previously PVACE2-treated cells revealed highest virus infection compared to untreated cells. An mRNA analysis in passages after PVACE2 infection was performed and showed non-stability of the overexpression throughout the passages, revealing a decrease of expression. The protocols used so far have been efficient in inducing an increase in ACE2 expression in the VERO E6, but this overexpression is not stable. Supported by PIBITI/CNPq-143059/2024-6 and CeVIVAS-FAPESP-2021/11944-6.

Keywords: Pseudovirus; SARS-CoV-2; Infection.

3243 - Category: VACCINES (VC)

Functional repercussion of an inherited IFNAR1 deficiency in a case of adverse event following yellow fever live-attenuated vaccination

GONÇALVES, B.D.S.¹; DA SILVA, A.M.V.²; MACHADO, L.¹; CUNHA, D.P.¹; AGONIGI, B.N.S.¹; COELHO, F.S.²; ROSA, L.C.²; MELGAÇO, J.G.²; TUBARÃO, L.N.²; DE OLIVEIRA, P.M.N.²; MAIA, M.D.L.D.S.²; BOM, A.P.D.A.²; BARROS, T.A.D.C.¹; VASCONCELOS, Z.¹. 1. INSTITUTO NACIONAL DE SAÚDE DA MULHER, DA CRIANÇA E DO ADOLESCENTE FERNANDES FIGUEIRA - IFF. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO DE TECNOLOGIA EM IMUNOBIOLOGICOS - BIO-MANGUINHOS. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Yellow fever virus (YFV) infection is a potentially fatal disease preventable by the live-attenuated YFV 17D/17DD vaccine. However, rare adverse events following immunization (AEFI-YF) have been linked to inborn errors of immunity (IEI) and autoantibodies against type I interferons (IFNs). Here, we aimed to perform a functional investigation of a structural *IFNAR1* deficiency identified in a Brazilian family with three siblings who developed AEFI-YF (one surviving proband and two deceased). The resultant protein was examined by molecular modeling, which revealed a deletion in a critical segment of the extracellular domain at the IFN- α 2B-binding site. Leukocytes from the proband and vaccinated controls were stimulated *in vitro* with YFV-17D, followed by multiplex cytokine quantification, immunophenotyping, and RNA sequencing. We also standardized two additional assays: phospho-STAT1 quantification by flow cytometry to assess *IFNAR1* function, and an enzyme-linked immunosorbent assay (ELISA) for anti-IFN autoantibodies. The proband's leukocytes showed baseline suppressed IFN responses. Upon YFV-17D stimulation, however, they produced high levels of type II IFN and exhibited robust activation of inflammatory and innate immune pathways. Profiling of the proband's innate and inflammatory responses revealed an inhibitory signature in NF- κ B signaling under both unstimulated and YFV-17D-stimulated conditions, alongside hyperactivation of inflammasome-associated genes upon stimulation. This study focuses on demonstrating the causal role of type I IFN signaling IEI in the pathogenesis of YFV-AEFI and deepening the mechanistic understanding of the type I IFN pathway by integrating familial investigations, functional assays, and molecular modeling. The phospho-STAT1 assay and anti-IFN ELISA are currently being standardized, which will help clarify this case and guide future research on similar events.

Keywords: Yellow fever vaccine; Adverse events following immunization (AEFI-YF); Inborn errors of immunity (IEI).

3249 - Category: VACCINES (VC)

Abnormal adaptative cellular immune responses in cases of VITT after COVID-19 vaccination

BARROS, T.A.D.C.¹; DA SILVA, A.M.V.²; PIAZI, M.D.¹; MENDES-DE-ALMEIDA, D.P.³; FACCION, R.S.¹; LOPES, M.R.G.¹; COELHO, F.S.²; ROSA, L.C.²; GONÇALVES, B.D.S.¹; DE ALMEIDA, C.D.²; DE OLIVEIRA, P.M.N.²; BORGES, M.B.J.²; VARGAS, F.R.⁴; MAIA, M.D.L.D.S.²; BOM, A.P.D.A.²; DE VASCONCELOS, Z.F.M.¹. 1. INSTITUTO NACIONAL DE SAÚDE DA MULHER, DA CRIANÇA E DO ADOLESCENTE FERNANDES FIGUEIRA - IFF. FIOCRUZ., RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO DE TECNOLOGIA EM IMUNOBIOLOGICOS - BIO-MANGUINHOS. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS - INI. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 4. INSTITUTO OSWALDO CRUZ - IOC. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Since the start of COVID-19 vaccination, rare cases of vaccine-induced immune thrombotic thrombocytopenia (VITT) have been reported. While the pathogenesis of VITT is still under investigation, the main hypothesis is that errors in the adaptive response lead to the hyperactivation of innate cells. Here we aimed to identify VITT biomarkers, by accessing the cellular response profile of a series of cases. For this purpose, peripheral blood mononuclear cells (PBMC) were isolated from 13 VITT cases (CAAE: 58916622.0.0000.5262), and seven healthy controls (CAAE: 34728920.4.0000.5262) followed by stimulation with SARS-CoV-2 Spike peptides, immunophenotyping and single cell RNA sequencing. Compared to healthy controls, the basal bulk transcriptomic response of VITT cases indicated activation of neutrophil degranulation, and thrombin signaling. Interestingly, it was observed an upregulation of peroxisome proliferator-activated receptor gamma (*PPARG*), T-box transcription factor 3 (*TBX3*) and downstream effector genes, associated to peripheral vascular disease. The Spike stimulation triggered a lower activation of T CD4+ (CD3+CD4+CD38+) and CD8+ (CD3+CD8+CD38+) lymphocytes compared to controls, as well as hyperexpression of genes related to STAT3 activation, and production of reactive oxygen species. The interferon signaling was downregulated in lymphocytes but not in monocytes in both basal and Spike-stimulated conditions. In conclusion, the VITT cases presented an abnormal cellular response profile, with T lymphocyte hypoactivation, associated to a lineage-specific downregulation of the interferon response. Further, VITT cases presented a baseline transcriptomic profile skewed for vascular inflammatory biological events, which may contribute to the clinical phenotype. Further investigation of VITT is necessary to uncover causalities, and to reduce vaccine hesitancy on immunization programs.

Keywords: Vaccine-induced immune thrombotic thrombocytopenia; COVID-19 vaccine; Single-cell transcriptomics.

3259 - Category: VACCINES (VC)

VACCINE HESITANCY IN BRAZIL DURING THE COVID-19 PANDEMIC: IMPLICATIONS FOR THE NATIONAL IMMUNIZATION PROGRAM

CAMARGO, I.C.R.C.; SILVEIRA, G.F.; BORDIGNON, J.. FIOCRUZ, CURITIBA - PR - BRASIL.

The COVID-19 pandemic posed unprecedented challenges to global public health. In Brazil, the National Immunization Program (PNI) faced not only logistical but also behavioral barriers, such as vaccine hesitancy and the spread of misinformation. This study analyzes the PNI's performance regarding COVID-19 by correlating vaccination coverage data with the population's informational behavior, monitored via Google Trends (GT). This is a descriptive and exploratory study with a quantitative approach. Official data on COVID-19 vaccination from 2019 to 2025 were collected, along with GT search terms. According to the Brazilian Ministry of Health, between 2019 and 2025, the country recorded a general decline in routine immunization coverage, while the COVID-19 campaign peaked with over 80% of the population receiving at least one dose — revealing contrasts in vaccine uptake. Terms with negative connotations, such as “vacina mata” (vaccine kills), peaked in January, March, May, and June — during periods when the Ministry of Health launched national campaigns, expanded school-based immunization, or announced new vaccine deliveries. In contrast, the term “vacina salva” (vaccine saves) remained consistently low. The neutral term “vacina covid” (COVID vaccine) also aligned with institutional actions: interest increased between February and April 2025, peaking after the “D-Day” and communication campaigns, but declined again in June and July, suggesting that engagement was temporary. These findings support the idea that, despite PNI efforts, a gap in public health communication limits campaign effectiveness. Integrating epidemiological and behavioral data reveals a mismatch between government actions and public perception. This may inform more effective, sustained, and context-aware communication strategies.

Keywords: COVID-19; Vaccination; Google Trends.

3266 - Category: VACCINES (VC)

Development of mRNA Vaccines for Parasitic Diseases: Leishmaniasis and Chagas Disease

CALDAS, G.D.A.B.. CTVACINAS/UFMG, BELO HORIZONTE - MG - BRASIL.

Despite considerable efforts by multiple research groups, the development of effective vaccines against parasitic diseases remains a major challenge. Given the technological breakthrough represented by mRNA vaccines during the COVID-19 pandemic, we chose to explore this platform for the development of a vaccine against Leishmaniasis. The kinetoplast-associated protein (LinKAP) was selected as a target antigen, having been identified through two-dimensional gel electrophoresis and mass spectrometry of *Leishmania amazonensis* proteins, using sera from mice immunized with the parasite's total protein extract. In previous work, we demonstrated that LinKAP confers partial protection against *L. infantum* infection when administered as a recombinant protein vaccine formulated with the adjuvant Poly ICLC. In the present study, we evaluated the protective potential of LinKAP delivered as an mRNA vaccine in a cutaneous leishmaniasis model. To assess its expression, HeLa cells were transfected with in vitro-transcribed, chemically modified LinKAP mRNA. Twenty hours post-transfection, LinKAP expression was confirmed by both immunofluorescence and Western blot analyses. Mice immunized with three doses of LinKAP mRNA encapsulated in lipid nanoparticles (LNP-LinKAP mRNA) produced high levels of antibodies and IFN- γ and showed partial protection against a challenge with *L. amazonensis*. A heterologous prime-boost-boost strategy using mRNA and protein also resulted in similar levels of protection, with no significant differences between groups regardless of the immunization protocol. In summary, our results highlight mRNA vaccines as an efficient and rapidly scalable platform for the development of vaccines against parasitic diseases, like Leishmaniasis.

Keywords: mRNA vaccines;Leishmaniasis;LNP.

3276 - Category: VACCINES (VC)

VaxLeish, a therapeutic vaccine for human visceral leishmaniasis based on a chimeric antigen with multiple tandem repetitive units.

DE OLIVEIRA, B.¹; ROQUE, M.C.¹; GOES, W.M.¹; FIORINI, A.C.¹; SALAZAR, N.¹; GOMEZ, D.¹; GOMES, I.P.¹; CARGNELUTTI, D.E.²; MOTTRAN, J.C.³; TEIXEIRA, S.M.R.¹; FERNANDES, A.P.¹; GAZZINELLI, R.T.¹. 1. CENTRO DE TECNOLOGIAS EM VACINAS (CTVACINAS - UFMG), BELO HORIZONTE - MG - BRASIL; 2. UNIVERSIDAD NACIONAL DE CUYO (UNCUYO), MENDOZA - ARGENTINA; 3. UNIVERSITY OF YORK, YORK - REINO UNIDO.

Human visceral leishmaniasis (HVL) is a life-threatening parasitic disease with no licensed human vaccine. To address this gap, we developed VaxLeish, a chimeric therapeutic vaccine composed of a novel mitochondrial antigen (LinKAP) fused to A2 protein and formulated with Poly ICLC. Immunoproteomics in protected mice vaccinated with *Leishmania* extract identified LinKAP, a Kinetoplast-Associated Protein-like from *L. infantum* (LinKAP), with conserved tandem repeats across *Leishmania* species. After differential centrifugation of *Leishmania* subcellular structures, LinKAP was enriched in similar fractions as found for mitochondrial proteins. This localization was confirmed by CRISPR-Cas9-mediated endogenous tagging and immunofluorescence imaging. CRISPR-Cas9 knockouts showed LinKAP is dispensable for parasite differentiation, infectivity, and replication. As a prophylactic vaccine, a truncated version (rLinKAP), sharing >85% homology among *Leishmania* species, induced strong Th1-biased responses and partial protection in mice and hamsters. To enhance efficacy, rLinKAP was fused to A2, an amastigote-stage antigen previously used in licensed veterinary vaccines, generating the chimeric rKAPA2 protein. We tested adjuvants in mice and dogs, identifying Poly ICLC as the most effective. rKAPA2 was produced under GLP, formulated with Poly ICLC (VaxLeish), and evaluated for immunogenicity, safety, stability, and therapeutic efficacy. As a therapeutic vaccine, VaxLeish promoted strong IFN- γ , low IL-10, and high IgG2a levels in infected mice and hamsters, reversing the immunosuppressive profile seen in controls. These responses correlated with reduced parasite burden. Toxicology studies in rats confirmed VaxLeish's safety and tolerability. Stability testing showed it remained stable for ≥ 90 days at 4°C and met quality standards. Together, these data support VaxLeish as a promising therapeutic vaccine for HVL and justify its progression to regulatory submission and clinical trials.

Keywords: Visceral leishmaniasis;GLP production;Therapeutic vaccine.

3281 - Category: VACCINES (VC)

Immunogenicity of *Staphylococcus aureus* recombinant proteins in association with granulocyte-macrophage colony-stimulating factor DNA vaccine in a murine model

DE SOUZA, F.N.¹; MEGGIOLARO, E.D.A.¹; ROSA, D.L.S.O.²; OLIVEIRA, A.C.D.¹; ANDRADE, L.A.F.³; SANCHEZ, E.M.R.¹; REIS, L.D.C.⁴; HEINEMANN, M.B.¹; GOTO, H.¹; GIDLUND, M.¹; LIBERA, A.M.M.P.D.¹; DA FONSECA, F.G.⁵; CERQUEIRA, M.M.O.P.⁵. 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSIDADE FEDERAL DE MINAS GERAIS, SÃO PAULO - SP - BRASIL; 4. UNIVERSIDAD NACIONAL TORIBIO RODRÍGUEZ DE MENDOZA DE AMAZONAS, CHACHAPOYAS - PERU; 5. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

Staphylococcus aureus is a major cause of bovine mastitis and a threat to public health due to its persistence, virulence, and limited response to treatment. In this context, the aim of this study was to evaluate the immunogenicity of recombinant SdrD, SdrE, and IsdH proteins combined with a GM-CSF DNA vaccine in a murine model. Sixty-three male C57BL/6J mice were divided into nine groups, each receiving a specific combination of the recombinant proteins SdrD, SdrE, and IsdH, either alone or in combination with the GM-CSF DNA vaccine. Lymphocyte proliferation and T cell subset distribution were assessed after *in vitro* culture of splenocytes under unstimulated and *S. aureus*-stimulated conditions, using BrdU assay and flow cytometry, respectively. Serum IgG1 and IgG2a levels against each antigen were measured by ELISA. Mice immunized with IsdH alone (group 3), the combination of IsdH, SdrD, and SdrE (group 4), or the same combination plus the GM-CSF DNA vaccine (group 5) showed increased lymphocyte proliferation. The group receiving the three proteins along with GM-CSF (group 5) exhibited the highest CD4⁺:CD8⁺ T cell ratio and a marked expansion of $\gamma\delta$ T cells after *S. aureus* stimulation, whereas all other groups showed a reduction in this population. Central memory CD4⁺ and $\gamma\delta$ T cells were also elevated in this group. All vaccinated groups developed strong IgG1 and IgG2a responses, with no additional antibody benefit observed from GM-CSF. The data demonstrate the potential of combining protein antigens with genetic adjuvants to stimulate both cellular and humoral immunity. These findings suggest that using multiple antigens in vaccine design may provide more effective protection against *S. aureus* infections than single-antigen approaches.

Keywords: T cell response; mastitis; *Staphylococcus aureus*.

3294 - Category: VACCINES (VC)

Immune response of the CoronaVac vaccine in a two-dose primary protocol and booster in risk groups.

OLIVEIRA, L.A.R.¹; CONTIN, S.V.M.¹; CORSINI, C.A.¹; MARTINS, P.F.D.S.¹; FILGUEIRAS, P.S.¹; LOURENÇO, A.J.¹; ALMEIDA, N.²; MARTINS-FILHO, O.A.²; NOGUEIRA, M.L.³; CASTILHO, L.²; DE OLIVEIRA, J.G.²; CARVALHO, A.T.²; CAMPOS, G.⁴; ALVES, P.A.²; FERNANDES, G.D.R.²; JEREMIAS, W.⁵; CAMPI-AZEVEDO, A.C.²; DOS REIS, J.G.A.C.²; CALVACANTE, C.C.⁶; CASTRO, L.L.M.E.²; QUEIROZ, R.F.G.E.⁷. 1. FUNDAÇÃO OSWALDO CRUZ- FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 3. FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO, SÃO JOSÉ DO RIO PRETO - SP - BRASIL; 4. FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO, SÃO PAULO - SP - BRASIL; 5. UNIVERSIDADE FEDERAL DE OURO PRETO, OURO PRETO - MG - BRASIL; 6. INSTITUTO BUTANTAN, SAO PAULO - SP - BRASIL; 7. FUNDAÇÃO OSWALDO CRUZ - FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

The CoronaVac inactivated virus vaccine played a role in Brazil during the pandemic particularly for risk groups like the elderly, children, adults, and adolescents. The aim of this study was to evaluate the immune response of antibodies and cells in these groups overtime, following both the primary vaccination schedule and a booster dose. A total of 2330 participants, aged 3 to 90, were followed from 2021 to 2023 consented and enrolled in the study. Blood samples were collected at one (1M), three (3M), six (6M), and twelve months (12M) after second dose in primary protocol (PP). The booster dose was administered after 6M. Analyses were performed to quantify the total IgG antibodies specific spike, neutralizing antibodies against BA.1 (VNT50) and biomarkers (Luminex). The $p < 0.05$ was considered significant. The sample included 558 (23.9%) children (5-11), 78 (3.3%) teenagers (12-17) 1446 (62%) adults (18-49) and 248 (10.6%) elderly (50-70). Following the booster, all groups showed a significant increase in IgG S at 12M compared to 6M. The seropositivity in children and teenagers was 100% at 6M, while adults and the elderly improved to 93.3% and 94.1% respectively, at 12M. Neutralizing antibodies against the BA.1 showed seropositivity of 100% in children and teenagers, 94.7% in adults and 81.8% in the elderly to 12M compared 1M. Regarding biomarkers, IL-6, TNF, VEGF e IL-2 were decreased after 12M compared to 6M. Others, like IL-1, IL-5, IL-10 e IL-13 also decreased in the elderly after 12M. In the children observed a decrease of IL-6 and G-CSF in 12M compared to 1M. The teenagers were found decreased of CXCL8, TNF and G-CSF in 12M compared to 3M. Meantime, the levels of these cytokines increased to 12M compared to 1M. In conclusion, vaccination proved effective for all groups. However, the results indicate that the cellular response in the risk groups decreases after 12 months, suggesting the requirement for additional booster doses to maintain protection.

Keywords: vaccine; CoronaVac; immune response.

3339 - Category: VACCINES (VC)

PRECLINICAL EVALUATION OF THE SAFETY AND EFFICACY OF A RECOMBINANT VACCINE AGAINST VISCERAL LEISHMANIASIS IN THE EXPERIMENTAL HAMSTER MODEL

PAITER, K.H.K.; VIANA, K.F.. UNIVERSIDADE FEDERAL DA INTEGRAÇÃO LATINO-AMERICANA (UNILA), FOZ DO IGUAÇU - PR - BRASIL.

Visceral leishmaniasis (VL) is a neglected zoonotic disease caused by *Leishmania infantum*, associated with high morbidity, mortality, and significant public health and economic impact in endemic regions. Current control strategies, including vector management and chemotherapy, face limitations such as high cost, toxicity, and the emergence of drug-resistant parasites. Despite decades of research, no vaccine with proven efficacy and broad applicability is commercially available, highlighting the urgent need for innovative prophylactic approaches. Glutamine Synthetase (GS) has been identified as a promising immunogenic target due to its role in parasite metabolism and its ability to elicit protective immune responses. This study aims to conduct a comprehensive preclinical evaluation of the safety, immunogenicity, and efficacy of a recombinant GS-based vaccine in a validated hamster model of VL, generating essential data to support future clinical trials in canine reservoirs. The vaccine will be developed using molecular cloning techniques for GS expression and purification. Hamsters will be immunized, experimentally challenged with *L. infantum*, and monitored for clinical and laboratory safety parameters. Humoral responses will be assessed by ELISA for specific anti-GS antibodies, while cellular responses will be evaluated through lymphoproliferation assays and IFN- γ quantification, indicative of Th1 polarization. Parasite burden in liver and spleen will be measured by qPCR to determine protective efficacy. This approach introduces an innovative vaccine strategy targeting an underexplored antigen, aiming to overcome current prophylactic limitations. Expected outcomes include demonstrating safety, absence of toxicity, and the induction of specific humoral and cellular immune responses in vaccinated hamsters prior to challenge, thereby supporting translational development for veterinary and, potentially, human application.

Keywords: Visceral leishmaniasis;recombinant vaccine;glutamine synthetase.

3343 - Category: VACCINES (VC)

Characterization of the Mechanisms of Protection for the Intranasal LaAg Vaccine Against Leishmaniasis

CORREA, G.A.D.C.; DA ROCHA, A.A.; ROMANO, J.V.P.; DE ALMEIDA, D.B.; ROSSI-BERGMANN, B.; MARTINS, A.M.D.F.; GUEDES, H.L.D.M.. FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Leishmaniasis is a group of diseases caused by protozoan species belonging to the genus *Leishmania*. Currently, there is no vaccine available to protect against *L. amazonensis* in humans. The vaccine candidate LaAg, a total lysate of *L. amazonensis*, provides partial protection when administered intranasally in C57BL/6 and BALB/c mouse models. In this study, we investigated the protective mechanisms of this vaccination. We vaccinated mice with LaAg or PBS (control) and infected knockout mice deficient in CD4, CD8, MHC II, IL-22, IFN- γ , IL-10, IL-23, or B cells (μ MT KO) with 2×10^5 parasites via subcutaneous injection. We monitored lesion size weekly and euthanized the animals at specific time points to assess parasitic load using the limiting dilution assay (LDA). The results showed that CD4 KO, IL-10 KO, MHC II KO, IL-22 KO, and IFN- γ KO mice had similar lesions and parasitic loads in both control and immunized groups. Immunization in CD8 KO, μ MT KO and IL-23 KO mice induced protection. These findings suggest that the vaccine's efficacy depends on IL-10, IL-22, IFN- γ , and CD4 T cells, but not on CD8 T cells, B lymphocytes or IL-23. Ongoing experiments aim to determine which cells express IL-10, IFN- γ , and IL-22 after immunization with LaAg and use cell transfer after sorting (mainly focused on CD4+ T cells) to identify the crucial cells for vaccine efficacy.

Keywords: Leishmaniasis;L; amazonensis;Vaccine.

3347 - Category: VACCINES (VC)

Characterization of ETRAMPs as vaccine candidates for vivax malaria

GOMES, C.B.D.S.¹; DE LACERDA, L.B.²; RODRIGUES, J.V.G.²; RODRIGUES, B.C.M.²; ROSA, M.J.G.²; JUNQUEIRA, C.F.². 1. CT VACINAS/ UFMG/ FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Malaria is the deadliest parasitic disease in the world, caused by protozoa of the *Plasmodium* genus and transmitted by mosquitoes. In Brazil, *P. vivax* is the predominant species with a significant impact on public health. Despite the different strategies used, no vaccine is licensed for this disease. Based on studies by our research group, a new protective mechanism was characterized involving cytotoxic CD8+ T lymphocytes in *P. vivax* infection, in addition to the identification of peptides presented by MHC class I of iRBCs. Among the identified antigens, the proteins PvETRAMP 4 and PvETRAMP 11.2 were selected for the present work. Both proteins were produced in a heterologous expression system in *E. coli* and purified by affinity chromatography. Next, C57BL/6 mice were immunized to assess immunogenicity of target antigens, by evaluating the antibody profile induced as well as the effector cellular response. Flow cytometry was used for immunophenotyping of CD4+ and CD8+ T lymphocyte populations. Finally, the animals were challenged with *P. yoelii* to evaluate the protective capacity of the tested antigens. The results obtained throughout the project reinforce the *in vivo* immunogenic capacity of the antigens in C57BL/6 mice, with a prominent TNF- α and IFN- γ response—cytokines classically associated with malaria protection—by CD4+ T cells, and a significant IFN- γ response by CD8+ T cells. Additionally, the proteins were able to induce a robust antibody response, the role of which should be further explored in future studies. Interestingly, upon heterologous challenge with *P. yoelii* 17XNL, only the PvETRAMP 4 protein demonstrated the ability to control parasitemia, with a reduction in blood parasite load from the seventeenth day of infection. Despite the promising findings, the protection studies indicate that further efforts employing transgenic parasites expressing the proteins of interest are necessary to confirm the efficacy of these antigens as vaccine candidates.

Keywords: vivax malaria;vaccine;ETRAMP.

3351 - Category: VACCINES (VC)

Non-clinical evaluation of the toxicity and safety of the Sars-COV-2 vaccine formulated by VLP

MARTINS, I.M.; GUARDALINI, L.G.O.; BERNARDINO, T.C.; QUINTILIO, W.; JORGE, S.A.C.; MARIA, D.A.. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL.

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has been a defining event of the 21st century, impacting global public health. Vaccines developed to combat Covid-19 are promising approach and the technology of virus-like particles (VLPs) that mimic the morphology of a virus and not contain genetic material, they are unable to replicate and without risk of infection. The aim of the study was to evaluate the safety and toxicity of the SARS-CoV-2 VLP vaccine using the recombinant baculoviruses (BVS, BVM, BVN, and BVE - encoding spike, membrane, nucleocapsid, and envelope gene proteins) in rats model as a promising strategy for the development of vaccines. Clinical monitoring of the animals was observed throughout of study, did not demonstrate behavioral changes due to the administration of the different vaccine antigens and adjuvant. It did not show quantitative differences in the number of red blood cells, hemoglobin, platelet quantity and biochemical changes between genders. Leukocytes were within normal parameters, despite the occurrence of differences in the total number of lymphocytes in female and eosinophils in male rats between the control groups and BVS particles. Histopathological analyses, pulmonary alterations were observed in both sexes, such as inflammation with a predominance of lymphocytes and plasma cells with few polymorphonuclear and macrophages mainly in the adjuvant, BVS particles and complete vaccine groups and the presence of bronchus-associated lymphoid tissue. In male rats, the presence of degeneration in the germinal epithelium was observed in the antigen fraction and complete vaccine groups, and in females vacuolization in the granulosa cell layer in BVS particles and complete vaccine groups. In addition, more studies will be needed to optimize VLP vaccines as an alternative for new viruses. This study was financed by Butantan Foundation and the São Paulo Research Foundation (FAPESP), Brasil. Process Number 2021/11946-9, 2024/06260-9.

Keywords: SARS-CoV-2;VLP;Toxicity.

3355 - Category: VACCINES (VC)

Evaluation of genetic variability and its potential impact on the antigenicity of B-cell epitopes of the Pv41 and PvGAMA protein in Brazilian isolates of *Plasmodium vivax*

ALENCAR, A.L.C.¹; CHAVES, L.B.²; DA SILVA, D.P.³; RODOLPHI, C.M.¹; SOARES, I.F.¹; BANIC, D.M.¹; MACHADO, R.L.D.¹; TOTINO, P.R.R.¹; DA SILVA, R.N.R.¹; DANIEL-RIBEIRO, C.T.¹; LIMA-JUNIOR, J.D.C.¹. 1. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. UFF, RIO DE JANEIRO - RJ - BRASIL; 3. UNIFASE, RIO DE JANEIRO - RJ - BRASIL.

Malaria parasites exhibit extensive genetic polymorphisms, many of which have evolved to evade host immune responses, posing a significant challenge to developing an effective malaria vaccine. In this context, the protein Pv41 (*Plasmodium vivax* Protein 41), a conserved surface protein expressed during the asexual (merozoite) stage, and PvGAMA (*P. vivax* GPI-anchored micronemal antigen), a GPI-anchored protein involved in parasite cell adhesion, has been identified as both antigenic and immunogenic. To investigate the genetic variability of these proteins and their potential impact on immune recognition, the study followed these steps: (1) amplification of the genes encoding Pv41 and PvGAMA from isolates obtained from 72 isolates of Pv41 and 24 isolates of PvGAMA different endemic regions of the Amazon - Belém (2), Mâncio Lima (10), Cruzeiro do Sul (10), São Gabriel da Cachoeira (21), Guajará (2), Rio Branco (5), Manaus (8), Oiapoque (7), Porto Velho (7) using polymerase chain reaction (PCR), followed by DNA sequencing via the Sanger method; (2) identification of B-cell epitopes using multiple immunoinformatics tools, including Bepipred, BCPREDS, and Vaxijen; and (3) analysis of the effect of antigenic variation in the most promising epitopes on the observed immune response. Analysis of the pv41 gene revealed both synonymous and non-synonymous mutations. Notably, one non-synonymous mutation occurred within the PEKQIDEHL epitope at position 170 (H170L) and A509T, affecting the antigenicity of this predicted epitope. In the N-terminal region of PvGAMA, two non-synonymous mutations were identified. Sequencing this region from samples collected in Porto Velho (9) and Manaus (15) revealed a single nucleotide polymorphism (SNP) common to both locations (95% prevalence). This SNP (T269C) represents a non-synonymous mutation that distinguishes the reference strain Sal-1 from the VCG-I strain (Colombia) and P01 strain (Papua Indonesia). Then, presented epitopes conserved.

Keywords: vaccine;vaccine targets;polymorphism.

3411 - Category: VACCINES (VC)

Differentially expressed genes in CD4+ and CD8+ T cells response in severe adverse events cases after COVID-19 vaccination

DA SILVA, A.M.V.¹; BARROS, T.A.D.C.¹; ROSA, L.C.¹; COELHO, F.S.¹; RODRIGUES, M.P.M.D.¹; DE ALMEIDA, C.D.¹; DE CARVALHO, C.²; LOPES, M.³; TUBARÃO, L.N.¹; MELGAÇO, J.G.¹; DE OLIVEIRA, P.M.N.¹; BORGES, M.B.J.⁴; FACCION, R.S.⁵; MENDES-DE-ALMEIDA, D.P.⁶; VASCONCELOS, Z.⁷; BOM, A.P.D.A.⁸. 1. INSTITUTO DE TECNOLOGIA EM IMUNOBIOLOGICOS BIO-MANGUINHOS, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO FERNANDES FIGUEIRA - IFF, RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO FERNANDES FIGUEIRA - IFF/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 4. INSTITUTO DE TECNOLOGIA EM IMUNOBIOLOGICOS BIO-MANGUINHOS - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 5. INSTITUTO FERNANDES FIGUEIRA IFF-FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 6. INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS, FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 7. INSTITUTO FERNANDES FIGUEIRA IFF - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 8. INSTITUTO DE TECNOLOGIA EM IMUNOBIOLOGICOS BIO-MANGUINHOS - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Vaccination is the most effective prevention against SARS-CoV-2, presenting a determining role to halt the spread by COVID-19. However, severe adverse events (SAE) following COVID-19 vaccination were reported, such as vaccine-induced immune thrombotic thrombocytopenia, Guillain-Barré syndrome, and others. Here we aimed to understand the mechanisms of SAE following COVID-19 vaccination by analyzing the immunologic profile of CD4/CD8 T cells. For this, peripheral blood mononuclear cells from 31 SAE cases (CAAE:58916622.0.0000.5262), and from 7 healthy controls, were stimulated using SARS-CoV-2 Spike peptides. Followed by immunophenotyping and cytokine quantification in cellular supernatant. Additionally, single-cell RNA sequencing was used to differentially expressed gene (DEG) determination. Compared to healthy controls, SAE participants had a lower frequency of activated CD8+T cells, along with decreased concentrations of IL-4, IL-6, IL-7, and GM-CSF in the supernatant. Upon stimulation, SAE participants exhibited 150 upregulated DEGs highlighting HLAs, CD19, CD79, and 378 downregulated DEGs in CD4+T cells. In CD8+T cells, 672 DEGs were upregulated, notably including IRF1, CCL4, RALB, and RAB3GAP1, while 882 DEGs were downregulated. Enrichment analysis of these DEGs demonstrated that CD4+T cells showed induction of pathways related to antigen presentation via MHC class II, B cell receptor signaling, and B cell activation. In contrast, genes in CD8+ T cells were associated with macroautophagy, interferon response, and regulation of vacuole organization. Individuals with SAE exhibited CD4+T cells with the expected cellular activation profile contributing to the adaptive immune response. However, the CD8+T lymphocytes present a dysfunctional response, associated with autophagic events. Our results suggest the impairment of T CD8 cells in SAE participants, an important antiviral immunological response, as a potential contributor to the SAE after COVID-19 vaccination.

Keywords: Severe adverse events;COVID-19 vaccine;Activated CD8+T.

3428 - Category: VACCINES (VC)

Inactivated Virus and Viral Vector COVID-19 Vaccines Depend Differentially on Humoral and Cellular Immunity to Confer Protection

RIGONI, T.D.S.¹; ABUNA, R.P.F.²; DO PATROCINIO, A.B.²; DE SOUZA, R.L.L.¹; RATTIS, B.¹; NONATO, O.C.D.S.¹; GAZZINELLI, R.T.³; CALADO, R.²; CABRAL-PICCOLI, M.P.⁴; BENEVIDES, L.⁵; SILVA, J.S.¹. 1. SÃO PAULO UNIVERSITY / FIOCRUZ, RIBEIRÃO PRETO - SP - BRASIL; 2. SÃO PAULO UNIVERSITY, RIBEIRÃO PRETO - SP - BRASIL; 3. FIOCRUZ, BELO HORIZONTE - MG - BRASIL; 4. A.C.CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 5. FIOCRUZ, RIBEIRÃO PRETO - SP - BRASIL.

Introduction: The efficacy of different COVID-19 vaccines has been demonstrated primarily through the induction of neutralizing antibodies. However, studies in mice have shown that vaccine-induced protection can occur even in the absence of neutralizing antibodies, mediated instead by T cell responses. Moreover, while the neutralizing capacity of antibodies is often reduced against emerging variants, T cell responses remain largely preserved. Human studies have demonstrated the induction of humoral and cellular response after vaccination. However, is not yet completely understood their role for the vaccinal protection. Thus, here we investigated the immunological mechanisms by which an inactivated virus (ViVac) or a viral vector (VvVac) COVID-19 vaccine confer protection. **Methods:** Transgenic mice expressing human ACE2 (k18-ACE2), as well as k18-ACE2 mice deficient in CD4⁺ T cells, CD8⁺ T cells or B cells, were immunized intramuscularly with two doses of each vaccine and challenged with SARS-CoV-2 virus. Survival and weight loss were monitored for 14 days. To evaluate the cellular response, splenocytes were cultured or not in the presence of the viral RBD protein, and CD4 and CD8 T cells responses were assessed by flow cytometry. The antibody response was also evaluated using neutralizing assays. **Results:** We observed that both immunizations conferred complete protection against SARS-CoV-2 infection. However, while VvVac induces a higher production of neutralizing antibodies, ViVac promotes a notable IFN- γ response and elicits a robust cytotoxic CD8⁺ T cells response. Interestingly, using Knockout mice, we found that the protection conferred by ViVac was lost in the absence of CD4⁺ T cells or B cells, whereas protection by VvVac was compromised only in the absence of B cells. **Conclusion:** ViVac relies on both T cell and B cell to confer protection, while VvVac depends only on the humoral response.

Keywords: Vaccines;T cells;COVID-19.

3453 - Category: VACCINES (VC)

Characterisation of the immunogenic properties of Triatoma virus VLPs for the development of vaccines against trypanosomatids

SOUSA-SILVA, M.¹; OLIVEIRA, A.D.N.¹; QUEIROZ, A.M.V.¹; GUÉRIN, D.M.A.²; VIGUERA, A.R.³. 1. POSTGRADUATE PROGRAMME IN PHARMACEUTICAL SCIENCES, IMMUNOPARASITOLOGY LABORATORY, DEPARTMENT OF CLINICAL AND TOXICOLOGICAL ANALYSIS, FEDERAL UNIVERSITY OF RIO GRANDE DO NORTE, NATAL - RN - BRASIL; 2. BIOPHYSICS INSTITUTE, UNIVERSITY OF BASQUE COUNTRY (CSIC-UPV/EHU), NATAL - ESPANHA; 3. BIOPHYSICS INSTITUTE, UNIVERSITY OF BASQUE COUNTRY (CSIC-UPV/EHU), BILBAO - ESPANHA.

Virus-Like Particles (VLPs) are viral protein structures widely used as adjuvants for formulating new vaccines. The immunoadjuvant properties of VLPs are associated with their ability to stimulate innate and acquired immune responses in a manner very similar to virus-induced immunity. In this context, the aim of this study was to evaluate the ability of VLPs to induce different types of IgG antibodies when administered together with recombinant antigens from *Trypanosoma cruzi* (the etiological agent of Chagas disease) or native antigenic extract from *Leishmania amazonensis* (the etiological agent of leishmaniasis). Additionally, we also want to evaluate the immunoadjuvant capacity of the structural proteins of VLPs, called VP1, VP2 and VP3. For this study, recombinant VLPs were produced in insect cells, and the recombinant proteins VP1, VP2, and VP3 were produced in bacteria. Subsequently, these VLPs and proteins were used in immunisation protocols in a murine model, and their immunoadjuvant properties were compared with adjuvants routinely used in immunisation protocols, namely Freund's adjuvant and aluminum salts. During the immunisation process, using different types of *T. cruzi* and *L. amazonensis* antigens, we observed that VLPs and the structural proteins VP1, VP2 and VP3 have the ability to polarize the antibody response, mainly specific antibodies of the IgG2a and IgG3 types, unlike the production of IgG1 and IgG2b antibodies. In conclusion, the ability of VLPs and VLP structural proteins to induce a polarized antibody response may have an important impact as potential adjuvants for the development of vaccines against trypanosomatids, since the mechanisms of resistance and susceptibility of these infections are associated with the mechanisms of polarization of the immune response.

Keywords: Virus-like particles;Adjuvant;Trypanosomatids.

2664 - Category: CELLULAR IMMUNOLOGY (CE)

Hypertension is associated with accelerated immunosenescence

TORRES, L.¹; VENTURA, L.H.D.A.¹; CAMATTA, G.C.¹; MOREIRA, F.C.¹; COELHO, M.M.¹; RAMIREZ, J.A.Z.²; ALMEIDA, M.¹; SATO, H.I.³; TEIXEIRA, S.M.R.³; TUPINAMBÁS, U.⁴; SILVEIRA-NUNES, G.⁵; MAIOLI, T.U.⁶; CARVALHO, A.T.⁴; DA FONSECA, D.M.⁷; FARIA, A.M.C.³. 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. USP, SAO PAULO - MG - BRASIL; 3. UFMG, BH - MG - BRASIL; 4. FIOCRUZ, BH - MG - BRASIL; 5. UFGV, GV - MG - BRASIL; 6. UFMG, GV - MG - BRASIL; 7. USP, SP - SP - BRASIL.

Hypertension is a global public health problem influenced by environmental, behavioral and genetic factors. In recent years, hypertension has been associated with chronic non-communicable diseases such as diabetes and obesity, and also with aging. However, little is known about the relationship between hypertension and cellular and immune senescence. Thus, this study aims to evaluate the correlation between the occurrence of hypertension, immunosenescence and responses to respiratory infections such as COVID and Flu-like syndromes. **Methods:** A total study sample of 309 volunteers was recruited. RT-PCR test for SARS-CoV-2 detection and viral load calculation was carried out to identify individuals with COVID-19. Peripheral blood mononuclear cells (PBMCs) were obtained in collected blood samples using heparinized vacuette by mononuclear cell isolation by Ficoll gradient (Histopaque—1077; Sigma) for flow cytometry of exhausted and senescent T cells. DNA from the PBMC samples was extracted according to the Trizol and chloroform protocol. Biological aging was assessed by integrating DNA methylation analysis into epigenetic clocks. **Results:** Comparing individuals with and without hypertension we identified changes in the plasma inflammatory profile with an increase in IFN γ and IL1-RA. In the immunosenescence phenotypic profile, we identified an increased in the frequency of exhausted and senescent CD8⁺ T cells in hypertensive individuals. Notably, individuals with hypertension had an increase in biological age when compared individuals without comorbidities (using the Horvath Updated epigenetic clock). In addition to these results, in our cohort, patients with COVID-19 and hypertension have a higher frequency of exhausted and senescent T cells, and longer hospital stay. The same is observed in patients with flu-like syndrome. Thus, our data show that hypertension is associated with an accelerated immunosenescence profile and a worse prognosis in respiratory infections.

Keywords: hypertension;Immunosenescence;epigenetic age

2692 - Category: CELLULAR IMMUNOLOGY (CE)

Surveillance of SARS-CoV-2 Immunogenicity and Virus-Specific CD8⁺ T Cells Reveal Fragility of Immunodominant HLA-A*02-Restricted Epitopes that Activate CD8⁺ T Cells

RIBEIRO, Á.L.¹; OLIVEIRA, P.D.M.¹; FERREIRA, G.M.¹; ARAÚJO, F.P.¹; DIAS, L.C.C.¹; LOURENÇO, A.A.¹; DE PONTES, L.G.¹; SANTOS, T.A.P.¹; ROCHA, V.D.M.¹; TEIXEIRA, C.W.¹; CLARINDO, F.A.¹; RETES, H.M.¹; BARBOSA-STANCIOLI, E.F.¹; DA FONSECA, F.G.¹; TSUJI, M.². 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. COLUMBIA UNIVERSITY, NEW YORK - ESTADOS UNIDOS DA AMERICA.

CD8⁺T cells are critical for antiviral protection against SARS-CoV-2, with less susceptibility to immune evasion. However, considering the evolution of SARS-CoV-2, these observations must be taken with care. Therefore, this study combined in silico and in vitro tools to evaluate HLA-A*02-restricted peptide signatures from variants of SARS-CoV-2 capable of triggering CD8⁺T-cell responses. Protein sequences from SARS-CoV-2 Wuhan virus and variants Alpha, Beta, Delta, Gamma, and Omicron were acquired in the NCBI Nucleotide database and used for prediction of HLA-A*02-restricted peptides with the software NetCTL. From this, 768 peptides were selected for in vitro evaluation of MHC-I-peptide reactivity using a peptide microarray and an HLA-A*02 recombinant molecule. Our results showed that non-structural proteins and the protein N are 'hot-spots' for the occurrence of HLA-A*02 reactive peptides, suggesting that these could be good targets for CD8⁺T cell response. Significant loss of immunodominant peptides from proteins S and ORF8 for all variants compared to the Wuhan virus was also observed, but reactivity for N peptides remained conserved. Next, peptides derived from S, N and RdRp proteins that were used to produce HLA-A*02 tetramers applied for the evaluation of the specific CD8⁺T cell response against SARS-CoV-2 in individuals HLA-A*02⁺ convalescents for COVID-19. Our assay revealed scarce memory CD8⁺T cell responses against the RdRp, but substantial cellular response against S and N proteins. Recall of S and N specific-CD8⁺T cells upon SARS-CoV-2 stimulation was also observed for convalescents, with a polyclonal profile. Taken together, these results confirm the importance of the immune response against the S protein, but also indicate that the N protein of SARS-CoV-2 could be exploited for the development of new vaccine platforms aiming at controlling viral dissemination.

Keywords: SARS-CoV-2;CD8⁺T cells;HLA-A*02-restricted peptides.

2798 - Category: CELLULAR IMMUNOLOGY (CE)

Epithelial proliferation triggered by inulin is mediated by $\gamma\delta$ T cells through microbiota remodeling and linked to anti-tumor activity

DE ASSIS, H.C.¹; PRAL, L.P.¹; RODOVALHO, V.D.R.²; REIS, B.S.³; DARCY, P.⁴; FACHI, J.L.⁵; MUCIDA, D.⁴; VINOLO, M.A.R.¹. 1. UNICAMP, CAMPINAS - SP - BRASIL; 2. NATIONAL LABORATORY FOR SCIENTIFIC COMPUTING, PETRÓPOLIS - RJ - BRASIL; 3. SANAVIA ONCOLOGY INC., NEW YORK CITY - ESTADOS UNIDOS DA AMERICA; 4. ROCKEFELLER UNIVERSITY, NEW YORK CITY - ESTADOS UNIDOS DA AMERICA; 5. WASHINGTON UNIVERSITY, ST. LOUIS - ESTADOS UNIDOS DA AMERICA.

$\gamma\delta$ T cells are responsive to microbiota and diet, essential for gut homeostasis and in responses to infections and cancer. Inulin fermentation promotes intestinal epithelial cell (IEC) proliferation depending on $\gamma\delta$ T cells. However, how these cells respond to inulin and its microbiota-signals remains unclear. This study aims to investigate the $\gamma\delta$ T cell response to inulin and its role to IEC proliferation during homeostasis and cancer.

Eight-week-old C57BL/6 wild-type (WT), *Tcrd*-GFP, and $\gamma\delta$ T cell-deficient (*Tcrd*^{-/-}) mice were fed control (AIN93) or inulin-enriched (AIN93+10%) diets for 4 weeks. Colonic cell proliferation (EdU⁺/Ki67⁺) was analyzed in histological sections. Stem cell function was assessed using intestinal organoids. Immune populations and cytokine production were analyzed by flow cytometry. For colorectal cancer (CRC) studies, *Cdx2*^{CRE-ER}*APC*^{flxed} (*iCdx2* ^{Δ APC}) mice were pretreated with the diets, received tamoxifen to induce tumors, and remained on diet for 4 more weeks.

Inulin promoted $\gamma\delta$ T cell expansion in colonic crypts and induced IL-17 production, without affecting IFN- γ or their proliferation. It also enhanced IEC proliferation in WT but not in *Tcrd*^{-/-} mice, indicating that $\gamma\delta$ T cells are required for this response. Loss of the phenotype in *Tcrd*^{-/-} mice correlated with a distinct microbiota, suggesting $\gamma\delta$ T cells shape it to support the response. Fecal supernatant from inulin-fed mice increased organoid formation, highlighting the role of microbiota-derived metabolites on the intestinal epithelium. In the CRC model, inulin reduced tumor burden in *iCdx2* ^{Δ APC} mice, which correlated with increased V γ 1 $\gamma\delta$ T cells and IFN- γ production.

These findings demonstrate that inulin-induced epithelial proliferation depends on $\gamma\delta$ T cells, likely through modulation of microbiota and microbial metabolites. Additionally, inulin enhances $\gamma\delta$ T cell-mediated antitumor responses in CRC, offering new insights into diet-immune-microbiota interactions in tumor regulation.

Keywords: microbiota;mucosal immunology;epithelium.

2801 - Category: CELLULAR IMMUNOLOGY (CE)

Maternal High-Fat Diet Alters Immune Lung Landscape in Neonatal Mice via ST2/IL-33 Axis Modulation

MENEGATI, L.M.¹; DE OLIVEIRA, E.E.¹; DE ABREU, A.M.²; DA SILVA, G.W.²; ALVES-FILHO, J.C.³; DA FONSECA, D.M.²; SILVA, F.M.D.C.E.¹. 1. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Introduction: Maternal obesity has been associated with increased risk of respiratory and inflammatory disorders in offspring. The IL-33/ST2/IL-13 axis, activated by the first breath, is important for post-birth homeostasis, leading to the differentiation of alveolar macrophages with an M2 profile by the recruitment of Innate lymphoid cells 2 (ILC) and consequent secretion of IL-13. However, remains unclear whether maternal obesity can influence this axis and the lung development of the offspring.

Aim: This study investigated the effects of maternal high-fat diet (HFD) on the pulmonary immune profile of neonatal mice, with a focus on the ST2/IL-33 axis.

Methods: Female C57BL/6 mice were fed a HFD for 8 weeks before mating with IL-33 reporter null males (IL-33^{gfp/gfp} C57BL/6 background), resulting in IL-33^{gfp/+} pups. Neonatal lung tissue was harvested at postnatal day 1 and analyzed by flow cytometry. Approved by CEUA ICB/USP 4117200623.

Results: Offspring from obese dams (IL-33^{gfp/+}) exhibited a marked increase in ST2 expression in the overall leukocyte population. Conversely, CD45⁺ cells showed a reduction of IL-33. Significant decreases were observed in the frequency of alveolar macrophages, eosinophils, and neutrophils. Notably, there was a sharp decline in regulatory T cells (Tregs) and CD8⁺ T lymphocytes. Innate lymphoid cells (ILCs) were also affected, with reductions in ILC1 and ILC2 populations, accompanied by an increase in ILC3 frequency and a slight upregulation of ST2 expression within this subset. Potentially impacting early-life immunity and susceptibility to respiratory diseases.

Conclusion: These findings suggest that maternal obesity reshapes the neonatal pulmonary immune environment through dysregulation of the IL-33/ST2 axis.

Keywords: maternal obesity;IL-33/ST2 axis;neonatal lung.

2897 - Category: CELLULAR IMMUNOLOGY (CE)

MerTk-mediated signaling boosts cigarette smoke lung inflammation mediating resistance to pneumococcal infection by modulating monocyte-derived alveolar macrophage

PINTO, K.G.; BRANDÃO, B.P.; DA COSTA, A.L.A.; FERREIRA, J.R.M.; MENDES, C.M.; MARINS, J.D.G.; NASCIMENTO, J.G.; SANTOS, J.M.; DE LIMA, J.V.C.S.; PIMENTA, A.J.; DE SÁ, M.C.M.D.M.A.; RIBEIRO, J.D.O.; FILARDY, A.D.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Smoking is a risk factor for pneumonia. Cigarette smoke (CS) induces airway inflammation and impairs immune responses, including the efferocytosis of apoptotic cells. The Axl and MerTk receptors mediate efferocytosis while simultaneously inhibiting pro-inflammatory pathways. Here, we investigated how Axl- and MerTk-mediated signaling affects the outcome of *S. pneumoniae* (SP) infection in mice previously exposed to CS. We first found high levels of nitric oxide in the bronchoalveolar lavage fluids (BALF) of CS-WT, CS-Axl^{-/-}, and CS-MerTk^{-/-} mice compared to the AIR groups. Although BALFs from CS-MerTk^{-/-} mice had increased numbers of total airway cells and neutrophils, we observed decreased numbers of resident alveolar macrophages (AMs) and monocyte-derived AMs (moAMs) compared to CS-Axl^{-/-} mice. Additionally, BALFs from CS-MerTk^{-/-} showed lower levels of TNF, IL-6, IL-10, IL-1b, and CXCL1 compared to CS-WT or CS-Axl^{-/-}. We also observed an increased number of dead neutrophils in the BALFs of CS-MerTk^{-/-} compared to CS-WT and CS-Axl^{-/-}. Following SP challenge, CSSP-MerTk^{-/-} mice exhibited higher mortality, bacterial loads and greater protein leakage CSSP-WT or CSSP-Axl^{-/-} mice. While SP translocation to the spleen was observed in all CSSP groups, we detected pulmonary commensals in the spleens of CSSP-MerTk^{-/-} mice. Moreover, BALFs from CSSP-MerTk^{-/-} mice had decreased numbers of total airway cells – including resident AMs, moAMs, and neutrophils - compared to CSSP-Axl^{-/-} mice. Importantly, BALFs from CSSP-MerTk^{-/-} mice showed lower levels of IL-1b and higher levels of IL-10 than CSSP-Axl^{-/-} mice. Finally, we found a greater number of moAMs expressing CD206 (M2-like) in CSSP-MerTk^{-/-} mice compared to CSSP-Axl^{-/-} mice. Overall, our findings suggest that MerTk-mediated signaling exacerbates CS-induced lung inflammation by modulating moAMs subpopulation fostering host protection against SP.

Keywords: Cigarette smoke;Efferocytosis;monocyte-derived alveolar macrophages.

2935 - Category: CELLULAR IMMUNOLOGY (CE)

The extracellular ATP sensor P2RX7 sustains the metabolic fitness of tissue-resident CD4⁺ T helper cells and promotes protective immunity against influenza reinfection

SANTIAGO-CARVALHO, I.¹; MACÊDO, B.D.G.¹; SALGADO, C.L.¹; LUBIANA, T.²; STOLL, C.R.¹; BIHNAM, D.¹; WHITE, E.¹; NAKAYA, H.I.³; LANCASTER, J.N.¹; LIMA, M.R.D.²; DA SILVA, H.B.¹. 1. MAYO CLINIC ARIZONA, PHOENIX - ESTADOS UNIDOS DA AMERICA; 2. UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL.

Developing a universal influenza vaccine requires understanding how lung-resident memory CD4⁺ T cells are formed. These cells remain in the lung for a long time and strengthen the immune response to secondary infections. Tissue-resident helper cells (TRH), a subset of memory T cells, share characteristics with follicular helper cells (TFH) and boost the IgA and IgG production by lung-resident B cells. TRH cells express elevated levels of the extracellular ATP (eATP) sensor P2RX7. Here we demonstrate that P2RX7 ablation on T cells impairs the establishment of flu-specific TRH cells. P2RX7-induced decrease of TRH cells negatively impacted the protection against homologous challenge, since T cell-specific P2RX7-KO mice exhibit worse control of viral load than WT mice. P2RX7-KO mice also showed a reduction in flu-specific lung-resident germinal center-like B cells and lower levels of IgA and IgG in the lungs. Notably, while B cells in WT lungs were broadly distributed and colocalized with CD4⁺ T cells, B cells in P2RX7-KO lungs formed iBALT-like clusters devoid of T cell help, and most were not flu-specific. P2RX7-KO did not induce major changes in the numbers of TFH-induced responses in the draining lymph nodes. Our single-cell RNAseq showed reduced expression of mitochondrial genes in P2RX7-KO TRH cells, which reflects decreased mitochondrial content presence in these cells. We observed an aberrant metabolic pathway usage by P2RX7-KO TRH cells, which beyond having less mitochondria also cease to utilize glycolytic metabolism to support protein synthesis. Our findings suggest the eATP-P2RX7 axis is required to establish TRH cells that protect against influenza.

Keywords: CD4⁺ TRMs;Influenza;P2RX7.

2944 - Category: CELLULAR IMMUNOLOGY (CE)

Contribution of microbiota and regulatory T cells for the protection against metabolic syndrome conferred by mucosal infection

OLIVEIRA, B.D.C.¹; DE SIQUEIRA, M.K.²; DA SILVA, G.W.¹; MENEZES-SILVA, L.¹; GONÇALVES, L.M.¹; DA CONCEIÇÃO, L.B.¹; LIMA, G.D.M.¹; RODRIGUES, G.M.B.¹; DE ARAUJO, M.V.¹; DE ASSIS, J.B.¹; MOREIRA, F.¹; SALGADO, C.L.³; AYUPE, M.C.¹; DA FONSECA, D.M.¹. 1. LABORATORY OF MUCOSAL IMMUNOLOGY, DEPARTMENT OF IMMUNOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES - UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. STANFORD UNIVERSITY, STANFORD - ESTADOS UNIDOS DA AMERICA; 3. MAYO CLINIC - ARIZONA, USA, PHOENIX - ESTADOS UNIDOS DA AMERICA.

Metabolic syndrome (MS) is a subgroup of conditions that encompasses obesity, type 2 diabetes, dyslipidemias, and metabolic-related liver diseases. Elucidating the mechanisms that influence the characteristics of MS is highly important for understanding the progression of these public-health challenging diseases. One event that can interfere with MS is gastrointestinal pathogens or pathogen-driven microbiota changes. After the clearance of acute infection with *Yersinia pseudotuberculosis* (YP), we showed that infected mice gained more weight and developed an inflammatory infiltrate and lymphatic dysfunction in mesenteric adipose tissue. Considering that both weight gain and chronic inflammation in adipose tissue are associated with the development of MS, we hypothesized that YP infection could interfere with and accelerate the development of MS. Our results show that previously infected mice displayed a greater inflammatory infiltrate in the liver, and increased bacterial translocation to the liver after infection clearance. Despite this, when fed the control diet, YP-infected animals have improved glucose clearance and insulin response in a microbiota-dependent phenotype. Similarly, previously infected mice are protected from a model of MS with a high-fat, high-fructose diet (NASH diet). When fed with the NASH diet, infected animals gain less weight and have a better glycemic response compared to uninfected mice on the same diet. They also respond better to insulin and have lower fasting glucose. Furthermore, these mice had higher levels of ketone bodies after a 6h fast. Our liver analysis shows that infected mice have protection against steatosis concomitant with an increase in parenchymal hepatic Tregs. These data open the possibility that the post-infection microbiota specific changes indirectly controls systemic metabolism by impacting the function and recruitment of regulatory T lymphocytes in the liver.

Keywords: Gut-liver axis ;Metabolism ;Regulatory T cells.

2956 - Category: CELLULAR IMMUNOLOGY (CE)

ROLE OF TRANSCRIPTIONAL CO-FACTOR IRF2BP2 IN ACTIVATION AND DIFFERENTIATION OF CD8 T CELLS

VIEIRA, B.L.M.D.O.¹; PEIXOTO, B.¹; CAMPOS, F.R.¹; PEREIRA, R.D.M.S.²; VIOLA, J.P.D.B.¹. 1. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Introduction: IRF2BP2 (Interferon Regulatory Factor-2-Binding Protein-2) is a transcriptional co-factor involved in the regulation of gene expression in different biological contexts. Our group previously described IRF2BP2 protein as a NFAT1 co-repressor playing a role in the activation and differentiation of T lymphocytes. To evaluate the role of IRF2BP2 in the CD8 T cell response *in vivo*, we generated a conditional transgenic mice that overexpress IRF2BP2 in T lymphocytes, LCK-Cre Rosa26 Stop-flox IRF2BP2-IRES-EGFP (IRF2BP2 TKi). **Results and Methods:** Naive IRF2BP2 TKi mice showed a reduction in T cell population in peripheral organs. IRF2BP2 TKi animals display increased percentage of CD8⁺CD44⁺ cells, which suggests that these animals present an increase of effector cells. To evaluate the effect of IRF2BP2 on CD8 T cell differentiation, we assessed effector and memory profile using *in vitro* skewed cell culture. Cells from IRF2BP2 TKi animals revealed an increased percentage of CD8⁺CD44⁺ population and an increase of granzyme B in effector cultures. In memory culture, cells from IRF2BP2 TKi animals revealed an increase of IFN- γ and Granzyme B. We assessed by qRT-PCR the master transcription factors involved CD8 T cell differentiation and observed an increase of *Tbet* and *Prdm1* in effector cells. These data suggested a positive regulation of some effector molecules by IRF2BP2 and suggest these cells present a phenotype of effector cells. We evaluated CD8 T cells response in a model of chronic viral infection with lymphocytic choriomeningitis virus (LCMV). The animals were challenged with sublethal dose of the LCMV, clone13. Twenty days after of infection, we observed a decrease of PD-1 in CD8 virus specific cells of IRF2BP2 TKi mice. These data suggest a reduction in the exhausted profile of these cells. **Conclusion:** Taken together, these data suggest a possible role for IRF2BP2 in regulating the balance between CD8 T cell activation and hyporesponsiveness.

Keywords: Irf2bp2;T CD8 Cells;Exhausted T Cells.

2962 - Category: CELLULAR IMMUNOLOGY (CE)

Amoxicillin-induced gut dysbiosis triggers a pro-inflammatory state that impairs reproduction – a possible gut–reproduction axis

SILVA, E.N.; AUGUSTO, L.V.; BORGES, M.P.; RAMOS, R.B.; DE ARAÚJO, L.P.; NOVAES, R.D.; DE ALMEIDA, L.A.; CORSETTI, P.P. UNIFAL-MG, ALFENAS - MG - BRASIL.

Health is strongly affected by the intestinal microbiota, which regulates the immune system. However, imbalances can lead to intestinal dysbiosis. So, this study investigated the relationship between the intestinal microbiota and reproductive health, examining the effects of dysbiosis caused by amoxicillin in mice and its impact on offspring. Female 129Sv/Ev mice were divided into two groups: 500mg/kg of amoxicillin (AMOX) and PBS for the same 15-day period. The oestrous cycle was evaluated by cytometry, cytology, and vaginal visualization on days 0, 5, 10, and 15. On the 15th day after the treatments, the fecal microbiota was evaluated by 16S rDNA metagenomics. Additionally, immunological parameters (*IL-1 β* and *IL-10*) and hormonal parameters (*AMH*, *FSH*, *FSH-R*, *LH*, *LH-R*, *Estradiol*, and *Progesterone*) were analyzed by gene expression in the caecum, ovary, pituitary gland, and histological analyses. Mice treated with AMOX showed alterations in the microbiota, causing dysbiosis and weight gain. Intestinal dysbiosis reduced the number of vaginal cells and affected the oestrous cycle. *IL-1 β* increased in the caecum and ovary, while *IL-10* decreased in these organs in dysbiotic mice. The oocyte area decreased, but the zona pellucida increased. The follicular teak also showed reduced thickness. Dysbiosis by AMOX too affected fertility and embryonic development, with a reduction in implantation rate and hormonal expression, compromising gestational success. Furthermore, offspring of dysbiotic mothers showed impaired survival, delayed body development, and disrupted intestinal microbiota composition. These results highlight the need to better understand the interactions between the intestinal microbiota and other physiological systems in relation to the action of antibiotics.

Keywords: Microbiome;immune response;animal reproduction.

3331 - Category: CELLULAR IMMUNOLOGY (CE)

Adipose tissue–derived small extracellular vesicles as key mediators of the immune response and tumor microenvironment modulation.

VENERANDO, L.A.F.¹; DIB, P.R.B.¹; JANUZZI, L.L.R.¹; JANUZZI, G.L.R.¹; SANTOS, M.D.O.¹; HOTTZ, E.D.¹; CANGUSSU, V.²; DE SOUZA, G.S.³; BASTOS, J.³; DO AMARAL, L.P.⁴; GAMEIRO, J.¹. 1. UNIVERSIDADE FEDERAL DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 2. HOSPITAL E MATERNIDADE THEREZINHA DE JESUS, JUIZ DE FORA - MG - BRASIL; 3. SANTA CASA DE MISERICÓRDIA DE JUIZ DE FORA, JUIZ DE FORA - MG - BRASIL; 4. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Obesity, characterized by the excessive accumulation of adipose tissue (AT) due to metabolic imbalances, impacts macrophage activation, which acquire distinct profiles depending on microenvironmental stimuli. Small extracellular vesicles (sEVs) from obesogenic visceral AT modulate macrophage polarization, promoting an inflammatory milieu linked to breast cancer progression. To investigate this interaction, we employed tumor cell models, macrophages from eutrophic and obese donors, and sEVs isolated from AT of bariatric surgery. These sEVs were characterized for size, morphology, vesicular markers, and phenotypic/biogenetic profile. Our data demonstrate that sEVs internally carry molecules associated with inflammation, platelet activation, and angiogenesis, with significant concentrations of CCL2, CD62P, and ICAM-1, indicating their modulatory potential in the tumor microenvironment. Preliminary results indicate that obesogenic sEVs activate the inflammasome, promoting caspase-1 cleavage and functional IL-1 β release in macrophages. To distinguish membranous versus intravesicular signals, we performed dot blot for caspase-1 and IL-1 β on intact and lysed sEVs; both preparations carried the pro-inflammatory pathway, confirming the sEV–macrophage assay data. M0 macrophages treated with sEVs from obese individuals exhibited increased nitric oxide and pro-inflammatory cytokines (IL-1 β , IL-6, IL-10, and TNF- α); M1 macrophages showed elevated TNF- α ; and M2 macrophages exposed to sEVs from eutrophic donors displayed increased IL-6 and IL-10. Obesogenic sEVs induced upregulation of CCL2 gene expression, whereas those derived from eutrophic individuals promoted IL-1 β upregulation. Tumor cell assays are in their final stages, and microRNA analyses and clinical data will be used for metabolic correlations (n = 6; ANOVA, p < 0.05). The origin of sEVs determines distinct inflammatory profiles with specific potential impacts on the tumor microenvironment.

Keywords: small extracellular vesicle;Macrophage;Tumor.

2728 - Category: CHEMOKINES AND TRAFFICKING (CT)

The CCL17–CCL22/CCR4 chemokine axis safeguards against obesity-induced metabolic imbalance

FRAGA JUNIOR, V.D.S.; DO NASCIMENTO, E.M.; DA SILVA, R.P.; NOGUEIRA, T.D.O.; BENJAMIM, C.F.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Background - Obesity is a chronic inflammatory disease strongly associated with metabolic syndrome and type 2 diabetes mellitus (T2DM). In this regard, adipose tissue hypertrophy leads to low-grade inflammation, driven by the accumulation of pro-inflammatory cytokines and immune cells. While chemokine receptors such as CCR2 and CCR5 have been linked to monocyte/macrophage infiltration and metabolic dysfunction, the role of lymphoid-expressed receptors remains poorly defined. CCR4 is a key chemokine receptor that regulates the trafficking and retention of Th2 and regulatory T (Treg) cells—two populations essential for maintaining adipose tissue homeostasis and immune balance. Tregs, in particular, secrete IL-10, which dampens inflammation and improves insulin sensitivity. Our study aims to explore the contribution of the CCL17–CCL22/CCR4 axis to the regulation of immune-metabolic interactions during obesity and its protective role limiting the progression of metabolic dysfunction.

Methods - To test our hypothesis, we subjected wild-type (WT) and CCR4-deficient (CCR4^{-/-}) C57BL/6J mice to a 45% high-fat diet (HFD) for 18 weeks.

Results - Analysis of adipose depots from HFD-fed WT mice revealed a marked upregulation of Ccr4 and its ligands at the mRNA level, but exclusively in brown adipose tissue ($p < 0.001$). On the other hand, CCR4^{-/-} mice on HFD exhibited greater weight gain and enhanced adipose hypertrophy index ($p < 0.001$), impaired glucose tolerance ($p < 0.05$) and increased insulin resistance ($p < 0.01$) compared to HFD-fed WT group, indicating that CCR4 deficiency adversely affects carbohydrate and lipid metabolism under hyperlipidic conditions. Liver histology showed lipid and glycogen accumulation in both WT and CCR4^{-/-} mice, with more pronounced alterations in the CCR4^{-/-} group.

Conclusion - Together, these findings indicate that CCR4 signaling impairment directly compromises metabolic function, enhances visceral adiposity, and worsens hepatic steatosis in obese mice.

Keywords: Obesity;Tregs;CCL17/CCL22-CCR4.

3142 - Category: CLINICAL IMMUNOLOGY (CL)

Validating a manufacturing process for CAR-T cells accessible to the Brazilian healthcare system for treating pediatric hematologic cancers

DOS REIS, S.A.¹; DOURADO, L.P.S.²; DE GODOY, D.T.²; ABDO, L.D.M.²; ARAGAO, E.A.A.²; NEUMANN, A.D.S.²; SILVA, L.R.B.²; BRAGA, F.H.P.²; KADAUKE, S.³; GRUPP, S.³; BONAMINO, M.H.². 1. INSTITUTO NACIONAL DE CANCER - INCA, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 3. CHILDREN'S HOSPITAL OF PHILADELPHIA, PHILADELPHIA - ESTADOS UNIDOS DA AMERICA.

Cellular immunotherapy has been transformed by the development of T lymphocytes genetically modified to express chimeric antigen receptors (CARs), enabling targeted recognition and elimination of tumor-associated antigens. Although CAR-T therapies are approved and commercially available in high-income countries, their implementation in Brazil remains limited due to high costs and infrastructure constraints.

To address this challenge, the Brazilian National Cancer Institute (INCA), in collaboration with the Children's Hospital of Philadelphia (CHOP), launched a technology transfer initiative to adapt CAR-T manufacturing for the treatment of pediatric hematologic malignancies within the Brazilian healthcare system. The strategy includes adapting existing laboratory infrastructure to comply with good manufacturing practices (GMP), using the CliniMACS Prodigy platform—a semi-automated, closed system for cellular processing.

The validation process employed leukapheresis material from a pediatric patient with acute lymphoblastic leukemia. T cells were purified, expanded, and transduced with a lentiviral vector encoding the CD19-BBz CAR. On the final day of culture, the CAR-T cell product was cryopreserved and subjected to quality control testing. Initial results demonstrated that the product met all predefined release criteria, including sterility, viability, transduction efficiency, absence of mycoplasma and replication-competent lentivirus, and a vector copy number below 8 copies per cell.

This work validates the feasibility of GMP-compliant CAR-T manufacturing in Brazil and represents a key step toward expanding national access to advanced immunotherapies through cost-effective and scalable solutions.

Keywords: CAR-T therapy;Pediatric leukemia;GMP manufacturing.

3317 - Category: CLINICAL IMMUNOLOGY (CL)

Innate-like T Cell Signatures in Oral Squamous Cell Carcinoma Reveal Links to Lymph Node Invasion and Disease Progression

OSHIRO, F.P.¹; GALDINO, N.A.D.L.²; SCARINI, J.F.¹; DE SÁ, R.S.¹; EVANGELISTA, G.F.D.B.¹; FIGUEIREDO, A.B.¹; KOHLER, H.F.²; DE OLIVEIRA, T.B.²; KOWALSKI, L.P.²; GOLLOB, K.J.¹. 1. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. A.C.CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

Oral squamous cell carcinomas (OSCC) arise at different sites within the oral cavity, usually from pre-malignant lesions. Although OSCC is one of the most common tumors, diagnosis often occurs at late stages. In addition to the low efficacy of treatment in advanced cases, typically benefiting only 50% of patients in stages III or IV, surgery is a major cause of morbidity, significantly impacting patients' quality of life and self-esteem.

In this study we collected blood, tumor and peritumoral samples from patients with stage III or IV OSCC. Blood samples were collected before surgery, 4 weeks after surgery and 6 months after adjuvant therapy. Cell samples were analyzed by multiparametric flow cytometry and plasma was analyzed with a multiplex bead assay. We evaluated blood, tumor and peritumor samples at the time of surgery, longitudinal changes during treatment, and clinical outcome, focusing on lymph node invasion at surgery and events of metastasis or relapse within 18 months or more.

Blood, peritumor and tumor exhibited distinct patterns of immune cell populations. While the blood was characterized by activated and CD3⁺TCR Va7.2⁺CD161⁺ cells, the tumor was enriched with PD-1⁺CD95⁺ or PD-L1⁺ cells and the peritumor had a mix of both populations, with an enrichment of TCRγδ⁺ and regulatory T cells. Interestingly, TCR Va7.2⁺ cells were enriched in patients with negative lymph nodes, while TCR Va7.2⁻ cells were common in patients with lymph node invasion. Conversely, relapse and metastasis were defined by the presence (or absence) of activated cells. In addition, patients without lymph node invasion or disease recurrence/metastasis had a small increase in the frequency of circulating TCRγδ⁺ cells.

These results highlight the importance of innate-like T cells in OSCC progression or resolution and may open new avenues for immunological research and therapy development.

Approved by the local Research Ethics Committee (#2532/18B). Supported by FAPESP and GSK (#21/00408-6).

Keywords: Oral squamous cell carcinoma;immune profiling;innate lymphocytes.

3449 - Category: CLINICAL IMMUNOLOGY (CL)

Metabolites increased after the reconstitution of milk microbiota are able to modulate the neonatal intestinal immunity in a colonization-dependent manner

SOVERAL, L.¹; FRIZZO, I.B.²; DE MELLO, I.M.A.¹; ESLABÃO, L.B.³; SONCINI, T.¹; AZEVEDO, P.N.⁴; GOMES, L.G.¹; PALMEIRO, J.K.¹; SINCERO, T.C.M.¹; IGNACIO, A.⁵; ROMÃO, P.R.T.⁶; MAIA, I.¹; KUPEK, E.¹; SORGI, C.A.⁴; ROMERO, O.B.¹; PIRES, M.M.D.S.¹; MCCOY, K.⁵; ZÁRATE-BLADÉS, C.R.¹. 1. UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRASIL; 2. UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANOPOLIS - SC - BRASIL; 3. UNIVERSIDADE FEDERAL SANTA CATARINA, FLORIANÓPOLIS - SC - BRASIL; 4. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 5. UNIVERSITY OF CALGARY, CALGARY - CANADA; 6. UFCSPA, PORTO ALEGRE - RS - BRASIL.

Human milk is the ideal nutrient for neonates, containing diverse metabolites, immune factors, cells, and a microbiota. Mother's own milk (MOM) is considered a form of personalized medicine due to individual variability in concentration of these components. When MOM is insufficient, pasteurized donor milk (PDM) is the best alternative; however, pasteurization alters molecular components and eliminates its microbiota. Cacho et al. (2017) showed in vitro that adding 10% MOM to 90% PDM and incubating at 37°C for 4 hours can partially reconstitute milk microbiota, producing reconstituted milk (RM). To evaluate its clinical feasibility, we conducted a trial with preterm neonates under 32 weeks gestation (ReBEC RBR-729kr8x). The intervention group (INT) received RM during the first 15 days of life, while controls (CON) received PDM only. No significant clinical differences or adverse events were observed. Fecal microbiota of INT neonates showed a more balanced Firmicutes/Bacteroidota ratio and found higher Bacteroides and Blautia in INT, linked to immune tolerance and gut health; while COM had more Pasteurellaceae and Haemophilus, associated with dysbiosis. Milk microbiota profiling showed RM microbiome resembled MOM more than PDM, with viable bacteria like Streptococcus and Lactobacillales reintroduced. Milk metabolomics revealed increased lysophosphatidylethanolamine 18:1 (LPE 18:1) and indoleacrylic acid (IA) in RM. The administration of these metabolites to germ-free mice increased tissue-resident macrophages and immune cells linked to Th1/Th17. Moreover, E. coli-monocolonized but not Akkermansia muciniphala monocolonized GF mice treated with the metabolites, showed reduction in tnfr-α and inf-γ gene expression in the gut. Our work shows that milk microbiota reconstitution and administration in the neonatal ICU is feasible, the procedure improves dramatically PDM nutritional quality and highlights the potential of milk metabolites as immunomodulators.

Keywords: Milk microbiota reconstitution;Preterm neonates nutrition;Milk metabolites immunomodulation.

3433 - Category: EDUCATION IN IMMUNOLOGY (EI)

BIOIMAGING BRASIL: DEMOCRATIZING INTRAVITAL MICROSCOPY ACROSS BRAZIL.

ANTUNES, M.M.¹; DE PAULA, C.¹; NETO, H.A.D.P.²; MENEZES, G.¹. 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Intravital microscopy (IVM) is a powerful tool that allows visualization of biological phenomena in vivo. However, widespread use of IVM is hindered by the lack of access to suitable equipment, and unfamiliarity with the technique and development of sophisticated microscopy systems has brought the assumption that IVM is unattainable. Therefore, an expansion in IVM application by Brazilian scientists could be achieved by reversing this logic. We developed a novel logistic strategy to empower institutions to establish IVM regionally: we bring IVM to them. **Aim, methods, results:** We visited 17 groups in 16 of the Brazilian states. This has been done by locally visiting different groups, which not only are exposed to presentational, open-access lecture of ~3h duration (basics of imaging techniques and principles), but also to practical exercises on mouse surgery and imaging procedures using their own microscopes. Also, we design custom-made, low-cost solutions and minor adjustments on their equipment at no cost to them (supported by our grant). Laboratories are selected by our team after registration in a virtual form, and women PIs from under-represented minorities have higher priority. In 03 years of project, we received 50 applications from 19 different states, from which 37 groups were selected. Of these, 17 groups have already been assisted in this period, totaling 639 trained scientists and 56,807 km travelled. **Conclusion:** Such unique initiative, supported by CZI Imaging Program, is generating a unprecedented democratization of IVM across Brazil, contributing to improve access to science and enhancing the quality of local projects. Also, based on the testimonials from visited members, we are witnessing a significant increase in scientific enthusiasm and drive for novel techniques. We hope that our initiative paves new roads for similar projects on other areas. **Grant:** Chan Zuckerberg Initiative – CZI.

Keywords: Intravital microscopy;Democratization of science;Immunology.

3085 - Category: HUMORAL IMMUNOLOGY (HI)

Uncovering Human Mucosal Immunity: Functional Profiling of Nasal B Cells

BECK, B.K.¹; JÚNIOR, A.B.D.C.¹; BRENO, F.D.M.¹; CAVALCANTE, S.D.F.¹; ASSIS, G.M.¹; RODRIGUES, D.A.S.¹; PEREIRA, C.F.D.A.¹; RODRIGUES, D.G.M.¹; CASTINEIRAS, T.M.¹; VICTORA, G.D.²; VALE, A.M.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. ROCKEFELLER, NEW YORK - ESTADOS UNIDOS DA AMERICA.

Respiratory viruses remain a global health challenge, highlighting the importance of understanding mucosal immunity. The nasal mucosa is a primary site of viral entry and a key location for immune protection, particularly through IgA responses that mediate viral neutralization and contribute to microbiota homeostasis. However, regulation of the local B cell repertoire remains poorly defined due to limited access to viable mucosal B cells. We hypothesize that respiratory infections reshape the nasal B cell repertoire by altering microbiota-reactive clones and potentially promoting autoreactive ones. These changes may result from local inflammation and lymphoid niches disruption. To investigate this, we optimized an ex vivo platform for phenotyping, sorting, single-cell culture, and BCR sequencing of B cells from nasal swabs and adenoids from infected and convalescent individuals. This approach yields ~1 million viable cells per swab. Flow cytometry revealed increased frequencies of memory B cells (MBCs) and plasmablasts/plasma cells in nasal samples compared to PBMCs, and access to germinal center B cells from adenoids. Over 70% of mucosal B cell subsets express IgA. While spike-specific MBCs represents ~1% of total MBCs in PBMCs, they were up to 20-fold enriched in the nasal mucosa of infected individuals, suggesting local clonal expansion or recruitment. These findings highlight a shift in nasal B cell repertoire and support the use of swabs to investigate local immunity. Ongoing analyses assess mucosal B cell reactivity to self-antigens and nasal commensals in homeostasis and infection. Our method enables functional and genetic profiling of local B cell responses in humans, with diagnostic and therapeutic potential. This platform opens avenues to explore how infections disrupt mucosal immune balance and supports development of vaccines targeting mucosal immunity.

Keywords: Mucosal immunity;B cells repertoire;Respiratory infection.

2644 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

IMMUNE SIGNATURES OF WILD-TYPE YELLOW FEVER VIRUS INFECTION: INSIGHTS FROM THE 2016–2018 BRAZILIAN OUTBREAK

GONÇALVES, A.P.¹; ALMEIDA, L.T.¹; FRADICO, J.R.B.¹; PASCOAL-XAVIER, M.A.¹; CALZAVARA-SILVA, C.E.¹; MONATH, T.²; LABEAUD, A.D.³; DRUMOND, B.P.⁴; CAMPI-AZEVEDO, A.C.¹; MARTINS-FILHO, O.A.¹; CARVALHO, A.T.¹; ALVES, P.A.¹. 1. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. QUIGLEY BIOPHARMA LLC, LEOMINSTER - ESTADOS UNIDOS DA AMERICA; 3. STANFORD UNIVERSITY, STANFORD - ESTADOS UNIDOS DA AMERICA; 4. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

The recent reemergence of sylvatic yellow fever in Brazil provided a unique opportunity to study immune responses to wild-type yellow fever virus (WT YFV) in naturally infected individuals. We characterized systemic immune responses in outbreak patients with different outcomes (death, hospital discharge, and late-relapsing hepatitis (LH)) and compared them to responses in 17DD vaccine recipients and seronegative controls. Serum samples collected at 1–15, 30–60, and 85–120 days post-infection were analyzed using a 48-plex Luminex assay to quantify circulating pro-inflammatory and regulatory cytokines, chemokines, and growth factors. Vaccinees were sampled 30–60 days post-vaccination. Neutralizing antibody (nAb) levels were assessed by plaque reduction neutralization tests. WT YFV infection triggered a robust, sustained inflammatory response, with significantly elevated soluble mediators compared to vaccinees and controls. Increased chemokines and growth factors indicated ongoing immune cell recruitment and tissue repair. Patients who died in the acute phase exhibited a highly dysregulated cytokine storm with elevated inflammatory and regulatory mediators. LH patients showed early elevations in IFN- α 2, IL-15, IL-2ra, IL-3, IL-4, IL-13, CCL2, CXCL8, G-CSF, and GM-CSF, with persistent or intensified patterns into convalescence, particularly among regulatory cytokines. WT YFV-infected individuals had higher nAb levels than vaccinees. Ongoing analyses will further explore links between soluble mediators, antibody responses, and clinical outcomes. Our findings advance understanding of WT YFV immunopathogenesis, distinguish natural from vaccine-induced responses, and identify potential biomarkers of disease severity and complications.

Keywords: Yellow fever virus;neutralizing antibodies;soluble mediators.

2651 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Immunization with *Trypanosoma cruzi* Trans-sialidase RNA sequences or recombinant proteins containing SAPA repeats protects mice against infection and promotes a balanced inflammatory response

DOS SANTOS, N.S.A.¹; JÚNIOR, C.R.D.A.¹; MEIRA, A.C.M.¹; CALDAS, G.D.A.B.²; FERNANDES, R.S.²; RICCI, M.F.¹; MELO, N.¹; DE CASTRO, J.T.²; SANCHES, R.C.D.O.¹; REIS-CUNHA, J.L.³; BARTOLOMEU, D.¹; OLIVEIRA, S.C.⁴; GAZZINELLI, R.T.²; MACHADO, F.S.¹; TEIXEIRA, S.M.R.². 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. UFMG/CT VACINAS, BELO HORIZONTE - MG - BRASIL; 3. UNIVERSITY OF YORK, YORK - INGLATERRA; 4. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL.

Proteins containing repeat domains are widespread among protozoa parasites. *Trypanosoma cruzi* has a family of surface proteins named trans-sialidases (TS), responsible for transferring sialic acid from host glycoconjugates to parasite mucins, which contains a C-terminal immunogenic domain with variable numbers of amino acids repeats known as SAPA (shed acute parasite antigen). Shed in the blood of infected animals and human patients, TS mediates several biological effects including the induction of immunosuppression during the acute stage of the infection. Here, we investigated the role of the SAPA domain of TS by immunizing mice with the recombinant full-length protein, TS without repeats, and a protein containing only the repeat domain as well as with RNA formulations encoding TS sequences with and without SAPA repeats. Besides confirming the immunodominance of the SAPA domain, after challenging the immunized animals with *T. cruzi* trypomastigotes, we showed that the presence of the repeats did not significantly impact the protection capacity of TS antigen during acute infection. However, immunization with TS protein or RNA containing the repeat domain resulted in increased production of IL-10 compared to mice immunized with RNA or recombinant protein without SAPA, and this increased IL-10 production correlates with a significant reduction in the inflammatory infiltrate in heart tissues of the infected animals. Altogether these results indicate that the SAPA domain plays a role in promoting an anti-inflammatory response that may contribute to increasing parasite survival. As a vaccine component, it can also contribute to promoting a balanced immune response.

Keywords: Immunization;Trans-sialidase;SAPA repeats.

2666 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Longitudinal Immune Response to a New Member of the Plasmodium vivax Erythrocyte Binding Protein Family (EBP2) in Individuals from the Brazilian Amazon

FERNANDES, G.M.¹; AFONSO, S.L.¹; MOREIRA-NASCIMENTO, S.L.¹; NASCIMENTO, M.F.A.¹; TORRES, L.M.²; CARVALHO, A.T.²; CAMPI-AZEVEDO, A.C.²; NTUMNGIA, F.B.³; ADAMS, J.H.³; KANO, F.S.¹; CARVALHO, L.H.¹. 1. MOLECULAR BIOLOGY AND IMMUNOLOGY OF MALARIA, RENÉ RACHOU INSTITUTE, OSWALD CRUZ FOUNDATION, BELO HORIZONTE - MG - BRASIL; 2. INTEGRATED RESEARCH GROUP ON BIOMARKERS, RENÉ RACHOU INSTITUTE, OSWALD CRUZ FOUNDATION, BELO HORIZONTE - MG - BRASIL; 3. CENTER FOR GLOBAL HEALTH AND INFECTIOUS DISEASES RESEARCH, COLLEGE OF PUBLIC HEALTH, UNIVERSITY OF SOUTH FLORIDA, TAMPA, FLORIDA, UNITED STATES OF AMERICA, TAMPA - ESTADOS UNIDOS DA AMERICA.

Currently, there are no vaccines available for malaria caused by *Plasmodium vivax* (Pv), the species responsible for most malaria cases outside Africa. The main vaccine candidate targeting the blood stage of Pv, the Duffy Binding Protein domain (DBPII), presents limitations, including high genetic polymorphism and strain-specific immunity. In this context, a second erythrocyte-binding protein from Pv (EBP2) has been investigated. EBP2 preferentially binds to young reticulocytes in the bone marrow, suggesting that may be involved in the extra-bloodstream parasite cycle. A worldwide genetic diversity of EBP2, demonstrated that region II of EBP2 is less polymorphic than DBPII, including in the Brazilian Amazon. Therefore, the immune response against EBP2 needs to be investigated. The experimental approach involved a 15-year follow-up study carried out among long-term Pv exposure Amazon individuals and included cross-sectional surveys at periods of high and low malaria transmission. Antibodies were measured using conventional ELISA assays. Specific cellular responses were evaluated by flow cytometry using commercial antibodies against different sub-sets of B/T cells. Overall, the results demonstrated that the frequency and persistence of anti-EBP2 IgG antibodies were significantly higher than those of anti-DBPII in all cross-sectional surveys. Unlike anti-DBPII antibodies, which fluctuated according to malaria transmission levels, anti-EBP2 responses remained stable over time. Antibody survival analysis revealed that 50% of DBPII responders lost their antibodies within 72 months, while 50% of EBP2 responders retained them for up to 180 months. Preliminary data on antigen-specific cellular responses indicated high frequencies of atypical B cells for both proteins. The sustained levels of anti-EBP2 antibodies reinforce the relevance of EBP2 in naturally acquired immunity, highlighting its potential as a key antigen in the development of a multivalent vaccine against Pv.

Keywords: *Plasmodium vivax*; Naturally Acquired Immunity; Erythrocyte Binding Protein.

2699 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

ANNEXIN A1 IS CRUCIAL DURING MURINE MODEL OF *T. cruzi* INFECTION, PREVENTING TISSUE DAMAGE, IMMUNE DYSREGULATION AND GUT MICROBIOTA IMBALANCE

OLIVEIRA, F.B.R.¹; RICCI, M.F.¹; OLIVEIRA, N.F.D.M.¹; RABELO, R.A.N.¹; SANTOS, L.L.D.O.¹; SANTANA, L.F.D.¹; SILVA, L.P.¹; BARBOSA, C.L.N.¹; DE FREITAS, G.M.¹; REZENDE, I.C.¹; DE ALMEIDA, V.M.²; ALVES, J.G.M.¹; TEIXEIRA, M.M.¹; COSTA, V.V.¹; CRUZ, J.D.S.¹; CASSALI, G.D.¹; MACHADO, F.S.¹. 1. UFMG, BELO HORIZONTE - MG - BRASIL; 2. FMRP-USP, RIBEIRÃO PRETO - SP - BRASIL.

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, leads to cardiac and digestive complications due to an imbalanced inflammatory response during the chronic phase, when current etiological treatments show little to no benefits. In this context, annexin A1 (ANXA1) plays a central role in resolving inflammation and restoring balance, though its role in DC persists unknown. Female BALB/c (WT) and ANXA1 knockout (ANXA1-KO) mice (8-11 weeks old) were infected (IF) or not with 10³ trypomastigotes (Y strain) and euthanized at 24 days post-infection (dpi). Additionally, IF-WT mice were treated from 17 to 23 dpi with Ac2-26 (ANXA1 mimetic peptide) or/and Benznidazole (Bz). Compared to IF-WT, IF-ANXA1-KO had increased parasitemia peak, significant weight loss and higher mortality rates, along with a worse clinical score. IF-ANXA1-KO exhibited reduced *Bacteroides* populations and elevated levels of aerobic lactic acid bacteria and Enterobacteriaceae. Moreover, IF-ANXA1-KO showed bacterial translocation into the blood and liver, which correlated with heightened intestinal vascular permeability. Histopathological assessments revealed higher scores in IF-ANXA1-KO mice's heart, colon and liver, accompanied by increased gut collagen fibers deposition and heart parasitism compared to IF-WT. Furthermore, cardiac function was significantly impaired in IF-ANXA1-KO mice. Although IF-ANXA1-KO presented an increased T-CD8⁺ IFN-γ⁺ (colon and heart) and T-CD4⁺ IFN-γ⁺ (heart) cells, these cells showed greater CTLA-4⁺ compared to IF-WT, suggesting immunological exhaustion. Notably, treatment with Ac2-26 and Ac2-26+Bz improved the clinical and the gut histopathological scores of IF-WT. Collectively, these results suggest that ANXA1 is a crucial regulator of CD pathogenesis, providing new perspectives on the disease's immunopathogenesis and suggesting ANXA1 as a potential therapeutic candidate, targeting *T. cruzi*-driven immune dysregulation. **Financial Support:** CNPq, Capes, FAPEMIG.

Keywords: *Trypanosoma cruzi*; Annexin A1; Immunoregulation.

2701 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Annexin A1 as a Therapeutic Target in Severe Malaria: Implications for Neuroprotection and Pulmonary Function

MELO, N.¹; RABELO, R.A.N.²; PEREIRA, R.D.D.¹; SILVA, L.P.¹; TEIXEIRA, S.L.¹; RICCI, M.F.¹; OLIVEIRA, F.B.R.¹; NONATO, A.¹; SANTOS, L.L.D.O.¹; BORGES, L.A.B.¹; SALVI, M.¹; COSTA, V.V.¹; QUEIROZ JUNIOR, C.¹; CASSALI, G.D.¹; TEIXEIRA, M.M.¹; GALVAO, I.¹; MACHADO, F.S.¹. 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL.

Malaria is the deadliest parasitic disease, with complications mainly caused by immune imbalance. Thus, it is important to investigate targets that regulate inflammation, such as Annexin A1 (AnxA1) and Suppressor of Cytokine Signaling 2 (SOCS2). Here, we investigate the role of AnxA1 in a murine model of severe malaria. BALB/c (WT) and AnxA1 knockout (KO) mice were infected (IF) with 10⁵ erythrocytes parasitized by *Plasmodium berghei* ANKA. Parasitemia, clinical score, histology, cytometry, lung function, immunohistochemistry, western blot and AC2-26 treatment were performed at selected time points. The absence of AnxA1 increased the susceptibility of IF mice, decreasing survival and worsened clinical scores compared to IF-WT. The infection increased immune cells infiltrate in the brain, mostly in IF-KO mice with a greater number of macrophages, neutrophils and dendritic cells, mainly producing TNF, and altered the proportion of adaptive immune cells, essentially TH17, compared to IF-WT. In the brain, SOCS2 expression occurred mainly in Purkinje neurons, the number of which decreased after infection, especially in KO mice. Despite this, SOCS2 expression was higher in IF-KO compared to WT. In the lung, a similar increased expression profile of SOCS2 was observed in IF-KO. In IF-WT circulating neutrophils, we detected an increase in coexpression and possible colocalization of AnxA1 and SOCS2, providing evidence of a relationship between them. We observed cytokine imbalance, lesions and changes in immune populations in the spleen, lung and liver of IF-KO mice. Notably, in these mice, lung function was worse compared to IF-WT. Furthermore, treatment of IF-WT mice with AC2-26, a mimetic peptide of AnxA1, protected against severe malaria, prolonging the survival rate (50%). Collectively, AnxA1 is critical for orchestrating the immune response, preserving neuro-pulmonary function, being an important target for severe malaria. Financial Support: Fapemig, CNPq and Capes.

Keywords: Malaria;Annexin A1;Immunoregulation.

2768 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Fiber-deprived diet weakens lung defense against antimicrobial-resistant *Klebsiella pneumoniae* and facilitates resistance phenotype in the gut microbiota

RICCI, M.F.. UFMG, BELO HORIZONTE - MG - BRASIL.

The rapid spread of antimicrobial-resistant bacteria is a major global health concern, worsened by the COVID-19 pandemic. While antibiotic misuse is a key contributor, infections with resistant pathogens also promote the spread of resistance genes, with microbial communities like the human microbiota serving as reservoirs. Disruptions in this balance—such as those caused by low-fiber diets—can increase the prevalence of resistance genes, heighten infection risk, and weaken host defenses. In this study, we evaluated how a low-fiber diet affects the gut microbiota resistome phenotype and examined its impact on susceptibility to lung infections caused by antimicrobial-resistant *Enterobacteriaceae*. In our study, dietary fiber deprivation induced significant alterations in the intestinal microbiota of C57BL/6 mice, leading to increased susceptibility to lung infection by the antimicrobial-resistant strain *Klebsiella pneumoniae* B31 (KP 31). Following lung infection, mice on a low-fiber diet exhibited elevated levels of ampicillin-resistant *Enterobacteriaceae* in the gut, along with notable shifts in overall microbiota composition. Importantly, germ-free mice that received fecal transplants from fiber-deprived donors also displayed heightened susceptibility to KP 31 infection, indicating that the altered microbiota plays a critical role in mediating this vulnerability. Infection further increased the abundance of cultivable resistant *Enterobacteriaceae* in the guts. *In vitro* assays suggest that propionate contributes to antimicrobial activity, and its reduction may facilitate the expansion of resistant bacteria. These findings suggest that gut microbiota serves as a key reservoir for resistance genes. Low-fiber diets can weaken lung defenses and promote resistome expansion after AMR infections. Understanding the factors that shape the intestinal resistome is essential for combat antimicrobial resistance.

Keywords: Microbiota;Inflammation;Antimicrobial-resistance.

2862 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Spatial profiling of human Diffuse Cutaneous Leishmaniasis lesions reveals immunosuppressive microenvironments that coincide with parasite localisation

DE CASTRO, C.H.D.F.¹; LOPES, P.D.O.¹; BARBOZA, B.D.A.¹; FREIRE-DE-LIMA, C.G.²; DECOTE-RICARDO, D.³; GUEDES, H.L.D.M.⁴; MARTINS, A.M.D.F.²; DE CARVALHO, L.P.⁵; BOAVENTURA, V.⁶; MOSSER, D.⁷; FALQUETO, A.¹; ALMEIDA, M.¹; COLES, M.⁸; AKBAR, A.⁹; BORGES, V.⁶; GOMES, D.C.D.O.¹; COVRE, L.P.¹. 1. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 4. FIOCRUZ-RJ, RIO DE JANEIRO - RJ - BRASIL; 5. FIOCRUZ-BA, SALVADOR - BA - BRASIL; 6. FIOCRUZ-BA, SALVADOR - ES - BRASIL; 7. UNIVERSITY OF MARYLAND, COLLEGE PARK - ESTADOS UNIDOS DA AMERICA; 8. UNIVERSITY OF OXFORD, OXFORD - INGLATERRA; 9. UNIVERSITY COLLEGE LONDON, LONDON - INGLATERRA.

Diffuse Cutaneous Leishmaniasis (DCL) is a severe infectious disease distinguished by *Leishmania amazonensis* protozoan persistent parasitism. DCL is characterised by multiple, non-ulcerated, nodular skin lesions with a profound immunosuppressive environment. We employed transcriptomic analyses and multiparametric immunofluorescence to assess the immune landscape of DCL lesions. We observed CD68+ phagocytes harbouring intracellular parasites, many of which displayed a characteristic vacuolated morphology. Spatial mapping of the lesions revealed distinct compartments in the skin which favoured vacuolated (VA+) or non-vacuolated (VA-) phagocytes. CD4+FOXP3+ regulatory T cells (Tregs) were significantly increase in VA+ regions and were found within the neighbourhood of CD68+ cells, suggesting a close association with an immunosuppressive microenvironment. Transcriptomic analyses of DCL lesions demonstrated that FOXP3, IL10 and LGALS1 transcripts were increased, along with a lower inflammatory transcript score when compared to localised cutaneous lesions. In addition, they also showed increased regulatory macrophages (regM) and decreased classically activated macrophages (M1) scores. Previously associated with anti-inflammatory responses, Heme catabolism pathway components were also markedly upregulated in DCL. This was validated by immunofluorescence staining, which confirmed the preferential location of CD68+ cells expressing heme oxygenase-1 (HO-1) within VA+ areas. Remarkably, the frequency of CD68+ cells expressing HO-1 showed a strong positive correlation with Treg frequencies, supporting the hypothesis of a coordinated immunoregulatory axis contributing to immune evasion and parasite persistence. Overall, our findings suggest the presence of an immunoregulatory niche within DCL lesions characterised by the co-localisation of parasite-ridden vacuolated macrophages and regulatory T cells that may reinforce the immunosuppression observed in this disease.

Keywords: Leishmaniasis;Multiparametric Immunofluorescence;Immunoregulation.

2914 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The role of DMT1 iron transporter in activation and differentiation of Th1 lymphocytes in Mycobacterium tuberculosis infection

DE ANDRADE, A.C.M.M.¹; CORREA, A.A.D.S.²; DENADAI, M.B.²; LOIOLA, L.H.M.¹; DE OLIVEIRA, J.¹; COSTA, D.L.¹. 1. USP, SÃO PAULO - SP - BRASIL; 2. USP, RIBEIRÃO PRETO - SP - BRASIL.

Tuberculosis (TB) is one of the diseases caused by a single infectious agent that kills most people in the world. A better understanding of the host response mechanisms that promote protection against *Mycobacterium tuberculosis* (Mtb) infection may help in the development of more effective strategies to cure TB. Iron plays an important role in both pathogen and host metabolism. T cells play a critical role in host resistance against Mtb infection, however, the role played by iron metabolism in the modulation of T cell function in TB is unknown. To investigate this, we first of all assess the influence of iron in Th1 differentiation *in vitro* isolating naïve TCD4 lymphocytes of DMT1^{fl/fl} (WT) or DMT1^{fl/fl} CD4 Cre⁺ (KO) mice to evaluate the differentiation and cytokine production of Th1 *in vitro*. Results shown a decrease in INF- γ ⁺ Tbet⁺CD4⁺ and TNF⁺Tbet⁺CD4⁺ expression, besides an increase in GMCSF⁺Tbet⁺CD4⁺ expression in KO in comparison to WT. Next, we co-cultured lymphocytes isolated from C7 mice (which express T cell receptors specific for ESAT-6) with Mtb-infected macrophages in the presence or absence of iron. The results indicated a lower bacterial load in the presence of iron. Finally, we infected C57BL/6 mice with H37Rv strain Mtb and evaluated the expression of DMT1 in pulmonary CD4⁺ T lymphocytes by flow cytometry. We found that the expression of DMT1 was increased in CD4⁺ T cells in response to Mtb infection, particularly in CD44⁺ and in Tbet⁺ CD4⁺ T lymphocytes at 4 weeks post-infection, indicating that they are upregulated in Th1 activated lymphocytes. Experiments to assess the difference in bacterial load in the lungs of KO compared to WT mice, as well as the overall evaluation of the adaptive immune response profile between them, are currently underway. Altogether, our results suggest that iron homeostasis in CD4⁺ T cells might be impacted by changes in DMT1 expression during Mtb infection, which may play a role in T cell function. Supported by FAPESP

Keywords: DMT1;Lymphocytes;M;tuberculosis.

2963 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Early immune signatures predict disease tolerance and resistance in COVID-19 progression

COBOS, E.V.M.¹; BASTOS, V.C.¹; DE LIRA, G.S.¹; SANTOS, C.M.R.¹; FERNANDES, H.D.D.P.¹; CONSTANCIO, C.D.S.¹; GAMA, A.M.D.S.¹; VIDAL, V.M.¹; RODRIGUES, D.A.S.¹; ALVES, L.S.¹; RENAULT, L.Z.¹; OTA, V.A.¹; DACRI, I.M.¹; TANURI, A.¹; JÚNIOR, O.D.C.F.¹; VALE, A.M.¹; CASTIÑEIRAS, T.M.P.P.¹; KAISERLIAN, D.²; DE LIMA, J.E.N.¹; BOZZA, M.T.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSITÉ CLAUDE BERNARD, LYON - FRANÇA.

The broad spectrum of clinical outcomes in SARS-CoV-2 infection—ranging from asymptomatic to lethal disease—poses a significant challenge. While severe inflammation in the lower respiratory tract has been linked to worse prognosis, other factors influencing viral load control (resistance) and the ability to mitigate tissue damage (disease tolerance) remain poorly understood, yet may play a critical role in COVID-19 progression. To address this question, we conducted a prospective study comparing the early immune responses of mildly symptomatic COVID-19 patients who later developed either (i) prolonged oligosymptomatic infection (beyond 21 days) or (ii) severe disease requiring oxygen supplementation. These groups represent distinct paradigms of low resistance with either high or low disease tolerance, allowing us to identify key molecular determinants of SARS-CoV-2 infection outcomes.

To this end, we collected nasopharyngeal swabs and peripheral blood samples from mildly symptomatic COVID-19 patients within the first 10 days after symptom onset, and performed multiplex cytokine analysis, flow cytometry, and RT-qPCR. Our findings revealed that mild-persistent infection was associated with reduced circulating NK cell counts, diminished type-I interferon responses, and lower levels of type 1 cytokines. In contrast, patients progressing to severe disease exhibited robust type 2 responses, along with strong activation of the antioxidant regulators NRF2 and HMOX-1. Notably, classic markers of disease severity—such as TNF- α , ferritin, and D-dimer—were not significantly elevated at this early stage, while soluble markers of tissue repair, including basic FGF and VEGF, were markedly lower in this group compared to other infected individuals.

These results suggest that type 2 cytokines (particularly IL-13) and impaired tissue repair are hallmarks of low disease tolerance in SARS-CoV-2 infection and may serve as early predictors of severe COVID-19 and therapeutic targets.

Keywords: COVID-19;Resistance;Disease Tolerance

2991 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Innate and adaptive immune signatures distinguish clinical presentations in a Malaria cohort revealed by high-dimensional flow cytometry

HORTA, L.¹; DE MENDONÇA, V.R.R.¹; LACERDA, M.²; KHOURI, R.¹; NETO, M.B.¹. 1. FIOCRUZ BAHIA, SALVADOR - BA - BRASIL; 2. FIOCRUZ AMAZONAS, MANAUS - AM - BRASIL.

Background: Understanding the immunological mechanisms that drive symptomatic versus asymptomatic *Plasmodium vivax* infection remains a critical challenge. We applied high-dimensional flow cytometry and tSNE analysis to profile PBMCs from a Brazilian cohort of 51 individuals: 17 uninfected controls, and 34 patients (16 asymptomatic, and 18 symptomatic), matched for age and sex. Two multiparameter panels (10 and 13 markers) targeting surface, cytokine, chemokine, and regulatory molecules were used to dissect immune heterogeneity in both CD3⁺ and CD3[−] compartments. tSNE visualization revealed distinct clustering of clinical groups, indicating distinct immune trajectories, particularly within the CD3[−] compartment. Symptomatic individuals showed an expansion of CD14⁺CCR2⁺ monocytes and CD16⁺CD56⁺ mature NK cells co-expressing STAT1 and CCR2. We also observed enrichment of CD56⁺IL-24⁺CD28⁺ICOS⁺CTLA4⁺ populations, activated CD56⁺TCR $\gamma\delta$ ⁺ subsets expressing IL-24 and CD28, and CD56⁺IL-24⁺CD16⁺ cells with high IFN γ R and CD64 expression. In CD3⁺CD4[−]CD8[−] cells, symptomatic individuals had increased CCR2 and CCR3 expression in TCR $\gamma\delta$ ⁺ cells and reduced TCR $\gamma\delta$ expression in CD8⁺ T cells, also among CD4⁺ cells elevated IL-6. In contrast, asymptomatic individuals retained higher TCR $\gamma\delta$ expression in CD8⁺ cells, suggesting a regulatory or protective role. **Conclusion:** These findings support the existence of two divergent immune trajectories in *P. vivax* infection. Asymptomatic individuals display a TCR $\gamma\delta$ ⁺CD8⁺-enriched profile suggestive of immune regulation, whereas symptomatic individuals exhibit a pro-inflammatory phenotype driven by monocytes, NK-like cells, and unconventional CD56⁺TCR $\gamma\delta$ ⁺ lymphocytes. Herein reported findings may aid in identifying biomarkers to distinguish asymptomatic and symptomatic malaria and guide the development of future immunomodulatory strategies. Keywords: malaria;tSNE;flow cytometry.

3020 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Ly6C^{high} Macrophages as the Main Inflammatory Cell in the Lungs of C3H/HeJ Mice in a Sublethal Model for Leptospirosis

LORGA, A.C.M.¹; DA SILVA, L.R.²; DOS SANTOS, L.B.R.²; COURROL, D.D.S.³; HEINEMANN, M.B.⁴; DA SILVA, L.F.F.⁵; AMAMURA, T.A.¹; ISAAC, L.². 1. ICB - USP, SÃO PAULO - SP - BRASIL; 2. ICB-USP, SÃO PAULO - SP - BRASIL; 3. INSTITUTO BUTANTÃ, SÃO PAULO - SP - BRASIL; 4. FMVZ - USP, SÃO PAULO - SP - BRASIL; 5. FM - USP, SÃO PAULO - SP - BRASIL.

Leptospirosis is a tropical neglected disease widely distributed around the world, causing nearly 1 million cases and 60,000 deaths annually. About 90% of infections are asymptomatic or mild, but severe forms such as Weil's syndrome and Leptospirosis-Associated Pulmonary Hemorrhage Syndrome (LPHS) can occur. Due to its nonspecific symptoms (fever, myalgia, cough), cases are likely underestimated. Pulmonary involvement is observed in up to 60% of patients, yet the mechanisms behind lung injury remain unclear, with hypotheses involving leptospiral toxins and/or immune dysregulation.

To investigate the pulmonary immune response during infection, male and female C3H/HeJ mice (3–5 weeks old), known to be susceptible to leptospirosis, were inoculated intraperitoneally with 3×10^8 *Leptospira interrogans* serovar Copenhageni L1-130 (n = 20) or PBS (control, n = 10). Seven days post-infection (d.p.i.), animals were euthanized for collection of bronchoalveolar lavage (BAL), lungs, kidneys, spleen, and serum. No lethality or significant weight loss was observed, suggesting a sublethal case of leptospirosis. The presence of leptospire in renal tissue was assessed by qPCR, confirming the infection. Histopathology revealed prominent lung inflammation without LPHS features. To characterize inflammatory cells (Ly6G⁺ granulocytes, MHC-II⁺ F4/80⁻ dendritic cells, and MHC-II⁺ F4/80⁺ macrophages) in the lungs, we performed a flow cytometry analysis employing a myeloid panel. This experiment indicated Ly6C^{high} macrophages — characterized as an inflammatory cell — as the main infiltrating leukocytes. However, no increased levels of TNF- α , IL-1 β , IL-6, or IL-18 were detected in serum, BAL, or lung. These findings suggested a sustained, but not prominent inflammatory response at 7 d.p.i.. The role of Ly6C^{high} macrophages in resolution and pathogenesis warrants further investigation to better understand the triggered immune response.

Keywords: Leptospirosis; Pulmonary Immune Response; Ly6C^{high} Macrophages.

3050 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

FABP-2 plasmatic levels and its relationship with cytokines during Plasmodium infection in individuals living in the Brazilian amazon

RODOLPHI, C.M.¹; OJEYINKA, D.I.²; SOARES, I.F.¹; ALENCAR, A.L.C.¹; BAPTISTA, B.D.O.¹; LEMOS, J.A.D.S.¹; PEREIRA, C.D.S.F.¹; DE SOUZA, H.A.D.S.¹; DE QUEIROZ, L.T.¹; MARTORANO, R.M.³; DE LUCA, P.M.¹; DA SILVA, R.N.R.¹; RIBEIRO, C.T.D.¹; RICCIO, L.R.P.¹; BERTHO, A.L.¹; GARCIA, A.B.²; CALJON, G.²; TOTINO, P.R.R.¹; LIMA JUNIOR, J.D.C.¹. 1. OSWALDO CRUZ FOUNDATION, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSITY OF ANTWERP, ANTWERP - BELGICA; 3. FEDERAL UNIVERSITY OF ACRE, CRUZEIRO DO SUL - AC - BRASIL.

Fatty Acid-Binding Protein 2 (FABP-2) is involved in fatty acid metabolism and is proposed as a biomarker of intestinal integrity and inflammation; however, little is known about its participation in inflammation during plasmodium infection. Therefore, we assessed serum FABP-2 levels in individuals with *P. vivax* and *P. falciparum* infections, investigating correlations with malaria history, genotype distributions, and inflammatory cytokine profiles. Participants were categorized into four groups: *P. vivax*-infected (PV), *P. falciparum*-infected (PF), endemic cohabitants (CH), and non-endemic controls (NEC). FABP-2 levels were measured via ELISA, and the Ala54Thr polymorphism was analyzed. Cytokine levels (IL-2, IL-4, IL-6, IL-10, IL-17A, TNF- α , and IFN- γ) were quantified. Our preliminary results indicate no significant differences in FABP-2 levels among groups (p = 0.2609) or based on malaria history. The Thr54 allele was present in 25% of participants, with genotype distributions following Hardy-Weinberg equilibrium (p = 0.485). The gastrointestinal symptoms in 54 infected individuals were not associated with FABP-2 concentrations. However, the cytokine profiling showed significant differences in IL-4 (p = 0.05), IL-6 (p < 0.001), and IL-10 (p < 0.001) among groups, with increased IL-6 and TNF- α in infected individuals. PCA indicated clustering of pro-inflammatory cytokines (TNF- α , IFN- γ , IL-6) in infected groups and a positive correlation between FABP-2 and IL-2. Moreover, FABP-2 correlated (p < 0.05) with IL-6 (r = 0.40, PV; r = 0.49, PF), TNF- α (r = 0.50, PV; r = 0.47, PF), and IL-10 (r = 0.38, PV), but not in the CH group. In conclusion, FABP-2 levels did not significantly differ among malaria-infected, endemic, and non-endemic individuals. However, the positive correlations with IL-6 and TNF- α suggest its participation in immune activation in infected groups. Further research is needed to clarify FABP-2's and other FABPs role in malaria-related inflammation.

Keywords: malaria; fabp2; cytokines.

3086 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Disease tolerant mice shelter a pathogenic intestinal microbiota able to trigger lethal disease in low tolerant hosts

VIDAL, V.M.¹; FERREIRA, L.M.¹; YILMAZ, B.²; COBOS, E.V.M.¹; GAMA, A.M.D.S.¹; KITOKO, J.Z.¹; ROSA, S.G.¹; TEIXEIRA, G.A.¹; FERREIRA, L.D.S.¹; NAZIOBERTO DUDA DE FARIAS, J.¹; RAGHAVAN, V.³; LITTMAN, D.³; DE SOUZA, H.S.P.¹; CANTO, F.⁴; BOZZA, M.T.¹. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSITY OF BERN, BERN - SUICA; 3. NEW YORK UNIVERSITY, NEW YORK - ESTADOS UNIDOS DA AMERICA; 4. UNIVERSIDADE FEDERAL FLUMINENSE, NITEROI - RJ - BRASIL.

Disease tolerance is a defensive strategy that limits tissue damage during infection. Macrophage migration inhibitory factor (MIF)-deficient mice (*Mif*^{-/-}) are protected in different models of infection and intestinal inflammation due to unclear disease tolerance mechanisms, whereas low disease-tolerant *Il10*^{-/-} mice develop microbiota-dependent spontaneous gut inflammation. We examined whether IL-10 is required for *Mif*^{-/-} mice phenotype and, conversely, the contribution of MIF during IL-10 deficiency. While breeding for double-deficient *Mif*^{-/-}*Il10*^{-/-} mice, we unexpectedly observed that *Il10*^{-/-} individuals died within days after co-housing with *Mif*^{-/-} mice. We found that healthy *Mif*^{-/-} hosts endure a highly diverse, unique and dysbiotic-like microbiota composition, including antibiotic-resistant *Enterobacteriaceae* species, which were sufficient to cause lethal Th1-driven colitis in *Il10*^{-/-} recipients. Mechanistically, *Mif*^{-/-} hosts exhibit reduced B cell frequencies in the intestinal mucosa and a community of bacteria largely uncoated by immunoglobulins - suggesting that MIF might influence microbiota composition through antibody-mediated regulation. In the absence of MIF, the colonic mucosa preserves morphofunctional integrity without overt inflammation, but displays a unique transcriptional profile marked by an increased expression of *Il22* and *EpiRegulin* – key promoters of barrier function. *Mif*^{-/-}*Il10*^{-/-} mice died prematurely, and survivors developed communicable disease, indicating lack of IL-10 as a dominant trait. Here, we identify MIF as a key regulator of the colonic microbial community, an inhibitory factor for disease tolerance and a promoter of tissue damage in the intestinal mucosa. Our data indicate that animals of tolerant genotype harbor a gut microbiota enriched in pathogens/pathobionts, which can trigger disease in susceptible individuals.

Keywords: MIF;Intestinal Inflammation;Inflammatory bowel disease.

3123 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Circulating and Tissue-Resident Memory T Cells Display Distinct Differentiation and Cytotoxicity Profiles in Localized Cutaneous Leishmaniasis

DE MOURA, R.G.¹; COVRE, L.P.¹; FALQUETO, A.¹; AKBAR, A.²; GOMES, D.C.D.O.³. 1. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITÓRIA - ES - BRASIL; 2. UNIVERSITY COLLEGE LONDON, LONDON - INGLATERRA; 3. UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, VITORIA - ES - BRASIL.

Localized Cutaneous Leishmaniasis (LCL) is a skin disease caused by *Leishmania braziliensis* that is driven by an exacerbated local immune response with a strong inflammatory and cytotoxic profile. Recent studies implicate immunosenescence as an additional contributor to the pathology, as patients accumulate senescent T cells in the blood that correlate with lesion size progression. Despite extensive analyses of circulating lymphocytes, the phenotype of T cells in the lesions is poorly defined. Tissue resident memory T cells (*T_{RM}*) act as sentinels in the skin and other organs, where they are readily poised to respond to antigenic challenges. While several studies have focused on characterizing *T_{RM}* in other skin diseases, their role in human LCL remains underexplored. To address this, we compared memory T cell subsets and *T_{RM}* in both peripheral blood and skin lesions. For in situ analyses, skin biopsies from healthy individuals and LCL lesions were collected, snap-frozen and immunostained. Moreover, PBMCs and lesional cell suspensions from the same donor were obtained and analyzed by flow cytometry. Our data shows that T cells differentiation states, though similar in the skin, diverge from their circulating counterparts. In addition, the majority of the lesional T cells do not express *T_{RM}* related markers, which could indicate that most lymphocytes infiltrating the skin originate from the circulation. Also, *T_{RM}* in the skin are predominantly CD69⁺ in both CD4⁺ and CD8⁺ compartments, with the latter being more associated with CD103 expression when compared to helper T cells. Functionally, both CD4⁺ and CD8⁺ *T_{RM}* are more cytotoxic (granzyme B⁺) than their non-*T_{RM}* populations. Collectively, this data indicates that T cells in the skin do not share the same differentiation profile and residence features as their circulating counterparts and that *T_{RM}* cytotoxic capacity could contribute to lesion formation, making them a potential target for future immunotherapies.

Keywords: localized cutaneous leishmaniasis;resident T cells;memory T cells.

3125 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

High glucose heightens vulnerability to *Leishmania braziliensis* infection in human macrophages by hampering the production of reactive oxygen species through TLR2 and TLR4

DA SILVA, I.B.S.¹; BASTOS, R.P.D.S.¹; ARAUJO, S.N.D.O.¹; DOS SANTOS, R.T.T.²; LAGO, A.¹; SILVA, J.¹; DE CARVALHO, L.P.¹; KHOURI, R.¹; ARRUDA, S.M.¹; BARRAL, A.M.P.¹; BOAVENTURA, V.¹; SEREZANI, C.H.³; DE CARVALHO FILHO, E.M.¹; BRODSKYN, C.I.¹; TAVARES, N.M.¹. 1. FUNDAÇÃO OSWALDO CRUZ, SALVADOR - BA - BRASIL; 2. UNIVERSITY OF CALIFORNIA, SAN FRANCISCO - ESTADOS UNIDOS DA AMERICA; 3. VANDERBILT UNIVERSITY MEDICAL CENTER, NASHVILLE - ESTADOS UNIDOS DA AMERICA.

Diabetes increases susceptibility to infections, including *Leishmania braziliensis* (*Lb*). Our group previously demonstrated that diabetic patients with cutaneous leishmaniasis (CL) take longer to heal lesions compared to non-diabetics. Since macrophages play a critical role in CL pathogenesis, we investigated how high glucose levels impact their response during *Lb* infection. Macrophages cultured in high glucose conditions showed increased parasite load than those in normal glucose conditions. The production of inflammatory mediators was similar between glucose conditions, but basal reactive oxygen species (ROS) production was elevated under high glucose conditions and remained unchanged after *Lb* infection, indicating glucose-induced oxidative stress does not control the parasite. In contrast, macrophages in normal glucose conditions, exhibited increased ROS production only after infection. Additionally, high glucose reduced TLR2 and TLR4 expression, which was also observed after *Lb* infection. TLR2/4 inhibition increased *Lb* infection in normal glucose conditions, mediated by TLR-dependent ROS production. However, this mechanism was absent under high glucose conditions, where elevated basal ROS production appeared TLR-independent. Biopsies from diabetic CL patients corroborated these findings, showing decreased TLR2 and TLR4 expression compared to non-diabetics. These findings suggest that high glucose levels induce oxidative stress and reduces TLR expression, impairing macrophage functions and rendering them less effective at controlling *Lb* infection. Keywords: Leishmaniasis; Diabetes; Macrophage.

3158 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

CCR4-Driven regulatory T cells migration shapes the outcome of *Leishmania* Infection

CHAGAS, G.G.¹; MENDES, M.T.²; BENEVIDES, L.³; COSTA, D.L.⁴; LIMA-JÚNIOR, D.D.S.⁵; SILVA, J.S.². 1. 1- DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY - FMRP - UNIVERSITY OF SÃO PAULO. 2- FIOCRUZ-SP, RIBEIRÃO PRETO - SP - BRASIL; 2. 1- FMRP - UNIVERSITY OF SÃO PAULO. 2- FIOCRUZ-SP, RIBEIRÃO PRETO - SP - BRASIL; 3. FIOCRUZ-SP, RIBEIRÃO PRETO - SP - BRASIL; 4. INSTITUTE OF BIOMEDICAL SCIENCES IV - UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 5. FMRP - UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Leishmania parasites induce the recruitment of leukocytes to the site of infection, a process that depends on chemokines and their receptors, such as CCR4. This receptor plays a critical role in cell migration by binding to its specific ligands and promoting chemotaxis of various immune cells, including regulatory T cells (Tregs). CCR4 is commonly expressed on the surface of Tregs, modulating the magnitude of the inflammatory response by regulating effector T cell proliferation and function, as well as the production of IL-10 and TGF- β . However, excessive Treg activity can impair protective immunity by suppressing effector T cell responses, ultimately allowing parasite persistence. Here, we hypothesized that CCR4 mediates Treg homing to infected tissues, thereby contributing to parasite survival. We, therefore, investigated the role of CCR4 in Treg migration to the dermal site and its functional impact in *Leishmania amazonensis* (*La*) infection. We found that CCR4 deficiency conferred increased resistance to *La*, with reduced skin pathology, inflammatory infiltrate and lower parasite loads. This enhanced resistance was associated with a pronounced effector T cell response and increased IFN- γ production in both draining lymph nodes (LN) and infected tissues. Notably, CD4⁺CD25⁺Foxp3⁺ Tregs failed to migrate to the infected dermis and draining LN in CCR4-deficient mice. These mice also exhibited reduced frequencies of Tregs expressing the co-inhibitory molecules GITR and CTLA-4. A reduction in CD4⁺IL-10⁺ cells was also observed at the infection site and in the ear-draining LN. In summary, CCR4-dependent Treg recruitment to *La*-infected dermal sites facilitates parasite persistence and disease progression. The observed reduction in pathology and parasite burden in CCR4-deficient mice highlights the potential of targeting the CCR4 axis to enhance protective immune responses. These results pave the way for future therapeutic strategies against cutaneous leishmaniasis.

Keywords: Regulatory T Cells (Tregs); CCR4; Immunoregulation.

3191 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The deleterious role of Gasdermin-D (GSDMD) in the control of *Toxoplasma gondii* by macrophages.

DE SOUZA, R.Q.¹; CANIATO, A.V.²; BORTOLUCI, K.R.². 1. UNIVERSIDADE DE SÃO PAULO (USP), SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO (UNIFESP), SÃO PAULO - SP - BRASIL.

Inflammasomes are cytoplasmic complexes that recognize pathogen-associated molecular patterns (PAMPs), cellular damage and changes in cell homeostasis. Upon recognition, these complexes undergo oligomerization, activating caspase-1, which cleaves pro-IL-1 β , pro-IL-18, and Gasdermin-D (GSDMD). GSDMD forms pores in the cell membrane, facilitating the secretion of IL-1 β and IL-18 and inducing pyroptosis, an inflammatory cell death. Inflammasomes are known to be activated during *Toxoplasma gondii* infection, playing crucial roles in controlling the infection. However, the role of GSDMD in this context remains unclear. The objective of this study is to evaluate the role of GSDMD during acute infection of *T. gondii* in macrophages. As expected, the spleen and peritoneum of Casp1/11^{-/-} mice exhibited higher parasite burdens compared to wild-type (WT) controls, whereas GSDMD^{-/-} mice showed lower parasite loads than both C57BL/6 and Casp1/11^{-/-} animals, suggesting a differential role of Casp1/11 and GSDMD during *in vivo* infection. Interestingly, GSDMD^{-/-} macrophages were less permissive to *T. gondii* replication compared to wild-type cells. This resistance did not appear to be related to Nitric Oxide release or IL-1 α production, known mechanisms involved in controlling *T. gondii*. In contrast, GSDMD^{-/-} cells exhibited higher IL-1 β production at early time points post-infection, which was mediated by NLRP3 activation. In accordance, pharmacological inhibition of NLRP3 in GSDMD^{-/-} cells reversed their resistance to *T. gondii* replication. Additionally, GSDMD^{-/-} macrophages display an increased number of viable cells and enhanced mitochondrial mass, suggesting that the differential activation of NLRP3 may result from increased availability of cells capable of assembling the inflammasome. Collectively, our results demonstrate that GSDMD may have a deleterious role in macrophage control of *T. gondii* through a mechanism that appears to involve a delay in NLRP3-mediated IL-1 β secretion.

Keywords: Inflammasome;Gasdermin-D;T; gondii.

3235 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

The differential role of Gasdermin D in murine astrocytes and microglia in response to *Toxoplasma gondii*

CANIATO, A.V.¹; DE SOUZA, R.Q.²; AMARAL, M.P.²; BORTOLUCI, K.R.¹. 1. UNIFESP, SÃO PAULO - SP - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL.

Inflammasomes are cytosolic sensors that detect pathogen- and damage-associated molecular patterns, triggering the activation of caspase-1 and the cleavage of IL-1 β , IL-18, and Gasdermin D (GSDMD), the key effector of pyroptosis. In the central nervous system (CNS), inflammasome activation may contribute to pathogen clearance but can also promote neuroinflammation and neurodegeneration. *Toxoplasma gondii*, the causative agent of toxoplasmosis, establishes chronic infection in the CNS through tissue cyst formation. While the role of inflammasomes during *T. gondii* infection has been extensively studied in immune cells, their consequences in the CNS remain poorly understood. Our results show that *T. gondii* activates inflammasomes in both microglia and astrocytes, as evidenced by ASC speck formation and release of IL-1 β and lactate dehydrogenase (LDH). In microglia, caspase-1 deficiency, but not GSDMD deficiency, led to increased parasite replication and reduced IL-1 β secretion. Similarly, IL-1R^{-/-} microglia exhibited a higher parasite burden, suggesting the caspase-1/IL-1R axis limits infection independently of GSDMD. Conversely, in astrocytes, the absence of caspase-1, GSDMD, or IL-1R conferred increased resistance to infection. This was not associated with impaired IL-1 β release, as both IL-1 α and IL-1 β were secreted through caspase-1- and GSDMD-dependent mechanisms. Notably, glycolysis inhibition reduced parasite replication in wild-type astrocytes, but it had no effect in caspase-1 or GSDMD knockouts, suggesting glycolytic metabolism is required for parasite replication and regulated by the caspase-1/GSDMD pathway. Pharmacological inhibition of GSDMD with disulfiram reduced parasite replication in astrocytes, highlighting GSDMD as a potential therapeutic target. These findings reveal a cell-type-specific role for GSDMD in controlling CNS *T. gondii* infection and identify metabolic regulation and GSDMD-dependent pathways as promising targets for host-directed therapy.

Keywords: Inflammasome;Toxoplasma gondii;Central Nervous System.

3304 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

DNA Extracellular Traps Released by Mayaro Virus-Infected Macrophages Act as a Platform for Virus Dissemination

DE CARVALHO, A.S.; DA SILVA, G.C.P.; ANDRADE, J.; FERREIRA, W.S.; WEISSMULLER, G.; SARAIVA, E.; DA POIAN, A.T.. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

Mayaro virus (MAYV) is an arthritogenic arbovirus that causes debilitating illness, potentially progressing to chronic rheumatic disease with persistent viral replication in joint tissue macrophages. Here, we report that MAYV-infected macrophages release decondensed DNA traps (DNA extracellular traps, DETs). This mechanism is driven by the production of reactive oxygen species (ROS) and the activation of peptidyl arginine deiminase (PAD), similar to neutrophil extracellular traps (NETs). However, unlike the typical pathogen clearance observed with NETs, MAYV-induced DETs did not inactivate the virus. Instead, DET-ensnared viruses are internalised by neighbouring uninfected macrophages, increasing the number of infected cells. This process is dependent on active viral replication, as an inactivated virus did not induce DET release. Furthermore, DET release proved to be dependent on viral load, as an increase in the multiplicity of infection (MOI) resulted in a corresponding increase in DET release. Disruption of DETs by DNase I decreased viral production and the percentage of MAYV-positive cells. Collectively, our findings suggest that MAYV-containing DETs act as a "Trojan horse" facilitating viral dissemination within inflamed tissues. This novel role for DETs connects macrophage-mediated inflammatory responses to viral persistence in articular tissue during chronic MAYV disease and offers significant implications for understanding MAYV infection pathology.

Keywords: DNA extracellular traps;Macrophage;Mayaro virus.

3307 - Category: IMMUNOLOGY OF INFECTIOUS AND PARASITIC DISEASES (ID)

Microbiota-Driven Inflammatory Imprints and Gut–Lung–Brain Axis Activation in a Murine Model of Long COVID-Like Pathology

LUCAS, J.M.¹; NETO, F.M.D.S.¹; DE JESUS, T.A.¹; FIGUEIREDO, E.P.¹; MAIELLO, M.S.¹; FRANÇA, A.S.¹; CUNHA, L.C.¹; MAGALHÃES, M.S.¹; LISBOA, S.F.¹; JÚNIOR, R.B.M.¹; BOAVENTURA, V.S.²; CARDOSO, C.R.D.B.¹. 1. USP - UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. GONÇALO MONIZ INSTITUTE - FIOCRUZ BAHIA, SALVADOR - BA - BRASIL.

Introduction: SARS-CoV-2 infection profoundly disrupts the intestinal ecosystem and in Long COVID (LC), gastrointestinal symptoms may persist alongside pulmonary and neurological sequelae. In a Brazilian cohort of 456 non-vaccinated individuals, 21.7% reported post-COVID diarrhea lasting a median of 86.8 days, thus underscoring the prolonged impact on gut homeostasis. **Objective:** We investigated whether microbiota altered by SARS-CoV-2 can drive systemic inflammation and behavioral dysfunctions characteristic of LC in a mice model. **Methods and results:** Hence, we performed fecal microbiota transplantation (FMT) from acute C57BL/6 K18-hACE2 infected mice into microbiota-depleted wild type recipients. Six days later, recipient animals displayed reduced hedonic responses, related with depressive-like behavior, and impaired memory, suggesting microbiota-induced neurobehavioral alterations. Next, to ensure gut susceptibility to later sequelae, mice were exposed, for another 6 days, to dextran sulfate sodium (DSS) colitis, which resulted in augmented clinical scores, increased IL-6 expression, and aggravated intestinal damage compared to controls. Strikingly, even in the absence of DSS, the FMT triggered robust expression of IL-1 β , IFN- γ , and IL-17 in gut tissues - surpassing levels observed in chemically induced colitis - revealing a potent pro-inflammatory capacity intrinsic to the dysbiotic microbiota. Moreover, lung tissues showed elevated inflammatory markers, particularly after colitis, reinforcing the contribution of the gut–lung axis to systemic inflammation. Finally, our findings demonstrated that SARS-CoV-2-induced dysbiosis alone is sufficient to promote intestinal, pulmonary, and neurobehavioral disturbances, recapitulating core features of LC. **Conclusion:** This model offers a novel platform to unravel microbiota-driven mechanisms in post-viral syndromes and highlights the gut microbiota as a therapeutic target in Long COVID.

Financial support: FAPESP, CNPq, CAPES

Keywords: Long COVID;Microbiota transplantation;Gut-lung-brain axis

2686 - Category: IMMUNOPHARMACOLOGY (IP)

A formulation based on an Annexin A1-derived peptide: An anti-inflammatory and pro-resolving therapy against severe dengue

MARTINS, J.R.¹; BATISTA, V.L.²; RAMOS, L.G.²; QUEIROZ JUNIOR, C.³; DA FONSECA, T.C.M.³; DIAS, A.S.L.⁴; SILVA, L.S.³; SANTOS, F.R.D.S.³; SANTOS, A.L.D.C.²; LULA, I.S.⁵; ROCHA, F.E.O.³; SOUZA, T.M.L.⁶; TEIXEIRA, M.M.⁷; GUIMARAES, P.P.G.⁸; COSTA, V.V.⁸. 1. DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, BRAZIL, BELO HORIZONTE - MG - BRASIL; 2. DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, BRAZIL, BELO HORIZONTE - MG - BRASIL; 3. DEPARTMENT OF MORPHOLOGY, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, BRAZIL, BELO HORIZONTE - MG - BRASIL; 4. EPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, BRAZIL, BELO HORIZONTE - MG - BRASIL; 5. DEPARTMENT OF CHEMISTRY, INSTITUTE OF EXACT SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, BRAZIL, BELO HORIZONTE - MG - BRASIL; 6. LABORATORY OF IMMUNOPHARMACOLOGY, OSWALDO CRUZ INSTITUTE, OSWALDO CRUZ FOUNDATION (FIOCRUZ), RIO DE JANEIRO, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO - RJ - BRASIL; 7. DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, BRAZIL., BELO HORIZONTE - MG - BRASIL; 8. DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS, INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS, BELO HORIZONTE, BRAZIL., BELO HORIZONTE - MG - BRASIL.

Severe dengue is characterized by intense inflammation, cytokine storm, vascular leakage, hemorrhage, and shock, largely mediated by the interaction between the host immune system and the dengue virus. Despite the disease's impact, there are no targeted therapies for dengue, highlighting the need for new therapeutic approaches. Our group identified Annexin-A1 as a key regulator in the immune mechanisms associated with dengue, where patients with severe dengue presented decreased plasma levels of the protein. Treatment with the Annexin-A1 mimetic peptide, Ac2-26, improved clinical and immunological markers in dengue-infected mice, without impairing viral control. However, systemic peptide use faces challenges like instability and rapid degradation. To address this, we developed a formulation of Ac2-26 with cyclodextrin (CDX-Ac2-26), which was characterized from a physicochemical and biological perspective to validate the construction of the formulation. CDX-Ac2-26 showed superior in vivo efficacy. In DENV-2-infected A129 mice (CEUA 26/2023), intraperitoneal administration of Ac2-26 or CDX-Ac2-26 (150 µg/mouse) reversed clinical scores and thrombocytopenia. Only CDX-Ac2-26 reduced mast cell degranulation, plasma MCPT-1, and splenic CCL2 levels. Notably, CDX-Ac2-26 retained efficacy at a threefold lower dose and via oral administration. Importantly, treatment with CDX-Ac2-26 did not alter viral titers, suggesting a selective pro-resolving and anti-inflammatory action. When combined with an antiviral drug, CDX-Ac2-26 completely prevented disease and mortality in infected mice, underscoring the relevance of combination therapies for associative effects, maximizing therapeutic efficacy against dengue with minimal side effects. This finding highlights the importance of developing peptide-based formulations that improves its stability and the value of adjuvant strategies targeting severe dengue. Keywords: Severe Dengue;Annexin A1;Cyclodextrin formulation.

2846 - Category: IMMUNOPHARMACOLOGY (IP)

Targeting Leukocyte Recruitment: Optimized CXCL9-Derived Peptides for Inflammation Control

DE OLIVEIRA, V.L.S.¹; BOFF, D.²; TEIXEIRA, M.M.²; AMARAL, F.A.²; PROOST, P.¹; STRUYF, S.¹. 1. KU LEUVEN, LEUVEN - BELGICA; 2. UFMG, BELO HORIZONTE - MG - BRASIL.

Inflammation is essential for pathogen control and tissue homeostasis, but excessive responses can lead to tissue damage. While anti-inflammatory drugs are widely used, they often lack specificity and are associated with limited efficacy and significant side effects. In this context, we developed a peptide derived from CXCL9, named CXCL9(74–103) or MIG30, which binds glycosaminoglycans (GAGs) and selectively inhibits leukocyte recruitment. This peptide showed anti-inflammatory effects in various murine models, including antigen- and MSU-induced joint inflammation, dinitrofluorobenzene-induced contact hypersensitivity, and lung inflammation caused by LPS, *Staphylococcus aureus*, *Klebsiella pneumoniae*, MHV-3, and SARS-CoV-2. Across models, CXCL9(74–103) reduced neutrophil infiltration and helped prevent tissue damage. However, the small size of the monomeric peptide results in rapid renal clearance, requiring frequent intravenous administration. To improve stability, we developed modified versions of the peptide. First, we generated a dimeric form to increase size and avidity. Second, we protected the N-terminus with pyroglutamic acid and proline (pQP), reducing aminopeptidase degradation. Third, we replaced L-amino acids with D-enantiomers to enhance resistance to proteolysis. These dimeric forms demonstrated increased stability: early administration reduced CXCL1-induced joint inflammation more effectively than the monomer, and high concentrations of the dimer remained detectable in the liver for extended periods even after subcutaneous injection. Additionally, while the monomer requires twice-daily administration to maintain therapeutic levels, a single daily dose of the D-dimer already showed promising effects in the *K. pneumoniae*-induced lung inflammation model. In conclusion, peptide dimerization and structural modifications substantially improved CXCL9(74–103) stability while preserving its anti-inflammatory function, supporting development for therapeutic use.

Keywords: CXCL9-derived peptides;GAG-binding peptides;Inflammation control.

2925 - Category: IMMUNOPHARMACOLOGY (IP)

Neutrophil Extracellular Vesicles as Mediators of Tumor Plasticity: a New Perspective in Cancer Immunotherapy

DE AMORIM, C.S.¹; DE AMORIM, N.S.¹; VALENÇA, L.D.S.¹; FRONY, A.C.²; DE MIRANDA, B.T.¹; ALMEIDA, P.P.²; DE-FREITAS-JUNIOR, J.C.M.²; RENOVATO-MARTINS, M.³; BARJA-FIDALGO, C.²; DE MORAES, J.A.¹. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. UERJ, RIO DE JANEIRO - RJ - BRASIL; 3. UFF, NITERÓI - RJ - BRASIL.

O câncer é uma doença complexa caracterizada pelo crescimento celular descontrolado e invasão tecidual. A inflamação, reconhecida como uma característica marcante do câncer por Hanahan e Weinberg (2011), desempenha um papel fundamental em sua progressão. Os neutrófilos foram observados pela primeira vez em tumores por Virchow em 1863 e são recrutados para o microambiente tumoral, onde podem auxiliar no processo de tumorigênese. Esses neutrófilos associados a tumores (TANs) exibem plasticidade e podem se polarizar em fenótipos N1 (antitumor) ou N2 (pró-tumor). Os neutrófilos N1 são citotóxicos, liberando mediadores pró-inflamatórios (p. ex., CD95, TNF- α , ICAM-1, NOS2) e gerando ROS por meio da atividade de NOX. Por outro lado, os neutrófilos N2 promovem a progressão tumoral expressando arginase-1, CCL2, VEGF, IL-8 e MMP-9. Nosso grupo foi o primeiro a polarizar neutrófilos humanos em um fenótipo semelhante ao N2 e investigar seu papel na promoção de tumores. Também testamos estratégias farmacológicas para reduzir a polarização de N2 e induzir a polarização de N1. Neste estudo, avaliamos como as vesículas extracelulares (VEs) liberadas por neutrófilos polarizados influenciam o comportamento das células tumorais. Os neutrófilos foram isolados de doadores saudáveis (CAAE 5372522.4.0000.5257) e estimulados por 3 h com LPS+IFN γ (N1) ou VEs-MDA (N2). Os fenótipos foram confirmados por citometria de fluxo (CD95+, CD184+) e as VEs foram analisadas por NanoSight. As VEs-N1 foram testadas na viabilidade de MDA-MB-231, MV3 e U87-MG via MTT. As EVs N2 foram avaliadas em MDA-MB-231 quanto à ativação de AKT, mitoROS (MitoSOX), migração (cicatrização de feridas), invasão (transwell), MMP-9 (zimografia) e atividade de NF- κ B (luciferase). A geração de EVs foi induzida por neutrófilos N1 e N2. As EVs N1 reduziram a viabilidade tumoral. As EVs N2 carregaram L-selectina, bloqueada por fucoidan, e aumentaram a mitoROS via PI3K/AKT. Também aumentaram a migração e a invasão via MMP-9 e NF- κ B. As EVs N1 foram antitumorais, enquanto as EVs N2 promoveram malignidade, posicionando-as como ferramentas ou alvos na terapia do câncer.

Keywords: Tumor-Associated Neutrophils; Extracellular Vesicles; Cancer.

3429 - Category: IMMUNOPHARMACOLOGY (IP)

Mycobacterial-induced lipid droplets function as an immunoinflammatory platform in DCs through DGAT-1 and PGE₂, contributing to M. bovis BCG burden

DA CRUZ, G.I.P.¹; DUTRA, F.S.P.²; TELLES, E.²; BOZZA, P.T.²; SILVA, M.F.D.S.C.³. 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL.

Tuberculosis (TB) represents a major challenge to public health. In the lungs, *Mycobacterium tuberculosis* (Mtb) infects macrophages and dendritic cells (DCs), which initiate the immune response. Mtb manipulates host lipid metabolism to survive and has triacylglycerol (TAG) as a carbon source to persist in a latent state in the host. Thus, we aimed to investigate the role of TAG synthesis in DC activation and bacterial burden during mycobacterial infection, focusing on the involvement of DGAT-1 and the eicosanoid PGE₂. BMDCs were differentiated from C57BL/6 bone marrow precursors with 10ng/ml of GM-CSF and infected with *M. bovis* BCG (MOI 5). BCG-infected DCs presented an increase in lipid droplets (LD), TAG and cholesterol ester (CE) accumulation, and lipid-related and proinflammatory genes expression. To evaluate the role of TAG synthesis, BCG-infected DCs were treated with DGAT-1 inhibitor (A922550). DGAT1 inhibition reduced bacterial burden, LDs accumulation, the proinflammatory genes *inos*, *il1b*, and *il6* expression, and the secretion of IL-1 β , IL-6, IL-10, IL-23, and TNF- α in DCs infected by BCG. We also observed that DGAT1 inhibition reduced the differentiation of IL-17-producing CD4⁺ T cells induced by the supernatant of infected DCs. On the other hand, A922500 treatment didn't modulate activation markers of infected DCs. Interestingly, PGE₂ secretion was also reduced by DGAT-1 inhibition on BCG-infected DCs. PGE₂ is an important inflammatory mediator of TB response and T cell differentiation. Then, PGE₂ was added to BCG-infected DCs and promoted an increased BCG burden, cytokine release, LDs accumulation, and in T cells CD4⁺ IL17⁺, also reverting the effects of DGAT1 inhibition. Together, TAG synthesis by DGAT1 and the lipid mediator PGE₂ are important to LDs formation, inflammatory response, and T cell differentiation by DCs infected by mycobacteria.

Keywords: tuberculosis ;lipid droplets;dendritic cell.

3480 - Category: IMMUNOPHARMACOLOGY (IP)

Inflammasome Inhibition by Modulation of Lipid Metabolism During SARS-CoV-2 Infection

SOARES, V.C.¹; DIAS, S.D.S.G.²; SANTOS, J.D.C.²; MOREIRA, I.B.G.¹; AZEVEDO-QUINTANILHA, I.G.²; BOZZA, P.T.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Viruses exploit host lipid metabolism for their replication, and the Sterol Regulatory Element-Binding Proteins (SREBPs) are a key regulator of fatty acid and cholesterol metabolism. SREBPs are also linked to lipid droplet (LD) biogenesis, a process our group discovered is induced by SARS-CoV-2 in human monocytes. Moreover, SREBPs are known to be involved in caspase-1 activation and pyroptotic cell death. However, their specific role during SARS-CoV-2 infection is largely unknown. We hypothesize that SREBPs regulate host metabolism, thereby influencing SARS-CoV-2 replication. To test this, we investigated the effect of pharmacological and molecular inhibition of SREBPs on lipid metabolism during SARS-CoV-2 infection. We infected human epithelial pneumocyte (Calu-3) cells with SARS-CoV-2 and found that infection leads to the expression and activation of SREBP1 and SREBP2. This activation upregulated genes involved in lipid metabolism and cytokine production, resulting in the accumulation of triglycerides, cholesterol, and the biogenesis of LDs. We observed a partial inhibition of both viral replication and cell death when either SREBP1 or SREBP2 was knocked down. A combined knockdown of both SREBP1 and SREBP2 showed a synergistic inhibitory effect, significantly downregulating lipid and cytokine genes, viral replication, and LD formation. Furthermore, we used the pharmacological inhibitor fatostatin, which effectively inhibited SREBP activation, and decreased the LD formation and viral replication. Notably, inhibition of both SREBPs and DGAT1 was found to block inflammasome activation by reducing the activation of caspase-1 and gasdermin D1, and release of IL-1 β and IL-18. In conclusion, our findings underscore the crucial role of SREBPs as master regulators during SARS-CoV-2 infection. Inhibiting these factors could be a critical strategy in combating viral infection by disrupting the metabolic pathways it exploits for replication and inflammatory responses.

Keywords: SARS-CoV-2;Inflammasome;Lipid droplets.

2804 - Category: IMMUNOREGULATION (IR)

Inhibition of Tonic PD-1 Signaling in Dendritic Cells Enhances Costimulatory Pathways

GASSEN, R.B.¹; LIMA, K.¹; BORGES, T.J.²; GARCIA, C.³; HUI, E.⁴; BONORINO, C.¹. 1. UFCSPA, PORTO ALEGRE - RS - BRASIL; 2. MGH, BOSTON - ESTADOS UNIDOS DA AMERICA; 3. STANFORD UNIVERSITY, STANFORD - ESTADOS UNIDOS DA AMERICA; 4. UCSD, SAN DIEGO - ESTADOS UNIDOS DA AMERICA.

Introduction: Immune checkpoint blockade therapies, particularly those targeting the PD-1/PD-L1 and CTLA-4 pathways, have fundamentally transformed cancer treatment, achieving clinical responses in approximately 25% of patients. Although combinatorial strategies have improved these outcomes, a substantial proportion of patients remain refractory to treatment or develop resistance. Emerging evidence implicates tonic PD-1 signaling—a ligand-independent, constitutive pathway distinct from canonical checkpoint inhibition—as a potential contributor to therapeutic resistance.

Objective: To elucidate the impact of tonic PD-1 signaling on dendritic cell (DC) function and to explore innovative therapeutic approaches aimed at restoring their immunogenic capacity.

Methods: We utilized lentiviral transduction to generate DC2.4 dendritic cell lines stably overexpressing PD-1. Under conditions of tonic PD-1 signaling, we evaluated cell viability and the expression of key costimulatory molecules (MHC II, CD80, CD86). Additionally, we investigated the effects of RIPR-PD-1, a bispecific molecule that disrupts tonic PD-1 signaling via CD45 tethering, and SHP009, a pharmacological inhibitor targeting SHP2 recruitment.

Results: PD-1 overexpression in DCs led to a significant reduction in both cell viability and the expression of costimulatory markers. Treatment with RIPR-PD-1 or SHP009 effectively reversed these inhibitory effects, restoring DC viability and promoting the upregulation of MHC II, CD80, and CD86 expression.

Conclusion: These findings demonstrate that tonic PD-1 signaling compromises dendritic cell functionality by attenuating their viability and costimulatory potential. Pharmacological blockade of tonic PD-1 signaling—either through RIPR-PD-1 or SHP2 inhibition—reinstates a pro-immunogenic DC phenotype. Targeting this non-canonical PD-1 pathway represents a promising strategy to potentiate antitumor immune responses and overcome resistance to existing immunotherapies.

Keywords: tonic PD-1 signaling;dendritic cell;immunotherapy.

2809 - Category: IMMUNOREGULATION (IR)

Transcriptional Repression of *Tnfa* and *Il12* by T-bet in Macrophages Attenuates Obesity-Driven Inflammation and Metabolic Dysregulation

DA COSTA, W.L.G.¹; TAVONI, T.M.²; SANTOS, W.G.S.²; CASTRO, G.¹; BATAGLIOLI, B.G.C.²; BERÇOT, M.R.²; SILVA, L.F.L.²; ANGELIM, M.K.S.C.³; REQUIAO, R.D.²; MARTINS, A.J.E.²; DOS SANTOS, T.P.²; FERRAIUOLO, H.C.C.¹; ANDRADE, J.N.B.²; SANCHEZ, L.R.B.²; VIEIRA, P.M.M.D.M.². 1. ICB - USP, SÃO PAULO - SP - BRASIL; 2. IB - UNICAMP, CAMPINAS - SP - BRASIL; 3. ST. LOUIS UNIVERSITY, SAINT LOUIS - ESTADOS UNIDOS DA AMERICA.

T-bet, a transcription factor known for its pro-inflammatory role via *Ifng* transactivation in lymphoid cells, has an underexplored function in myeloid cells, especially macrophages. We previously showed that myeloid-specific *Tbx21* knockout(mKO) in C57BL/6J worsens inflammation and decreases survival in bacterial sepsis, indicating a protective role of macrophage T-bet in acute inflammation. Aiming to explore its role in chronic metabolic inflammation, we found increased *Tbx21* expression in adipose tissue macrophages(ATMs) from obese mice by scRNA-seq. mKO mice fed with both chow or high-fat diet had increased food intake(kcal) but reduced body weight(g), with lower lean and unchanged fat mass(Nuclear Magnetic Resonance). Thermal imaging(FLIR) showed higher interscapular temperature in mKO mice, suggesting increased BAT thermogenesis, confirmed by UCP1(WB). mKO mice exhibited elevated fasting glycemia, glucose intolerance(GTT), insulin resistance(ITT), and higher serum TNF- α . Histology revealed smaller adipocytes and reduced hepatic steatosis. Flow cytometry of perigonadal adipose tissue(pgAT) showed increased inflammatory infiltrate in mKO mice, with higher TNF α ⁺/IL-12⁺ ATMs, and pgAT lymphoid cells had more IFN γ , suggesting disrupted inflammation control. T-bet-deficient Bone Marrow-Derived Macrophages (BMDMs), stimulated with palmitate 0.4mM, insulin 10nM, and glucose 30mM (MME), showed increased *Tnfa* and *Il12* expression(qPCR), cytokine secretion(ELISA), glycolytic shift(ECAR), and impaired OXPHOS(OCR). ChIP confirmed T-bet binding at *Tnfa* and *Il12* promoters, indicating transcriptional repression. Conditioned medium from *Tbx21*KO MME impaired insulin-stimulated AKT SER473 phosphorylation(WB) in 3T3-F442a adipocyte-like cells and human hepatocytes HUH7. These findings reveal an anti-inflammatory role for macrophage T-bet, contrasting its pro-inflammatory role in lymphoid cells, and highlight it as a potential target for immunometabolic therapies in type 2 diabetes.

Keywords: *Tbx21*/T-bet;Macrophages;Metabolic Dysfunction.

2831 - Category: IMMUNOREGULATION (IR)

mitoKATP Channel Suppression Drives LPS Tolerance via ROS-Mediated HIF-1 α /NF- κ B and Mitochondrial Hyperfission

SILVA, L.F.L.¹; ANGELIM, M.K.S.C.²; REQUIAO, R.D.²; DA COSTA, W.L.G.²; WATARI, K.³; DE CASTRO, G.⁴; CAMPOS, A.T.P.⁴; ZANI, N.C.⁴; TEÓFILO, F.B.S.²; KARIN, M.³; ABDULKADER, F.R.D.M.⁴; VIEIRA, P.M.M.D.M.². 1. UNICAMP, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE CAMPINAS, CAMPINAS - SP - BRASIL; 3. UNIVERSIDADE DE CALIFÓRNIA, SAN DIEGO, SAN DIEGO - ESTADOS UNIDOS DA AMERICA; 4. UNIVERSIDADE DE SÃO PAULO, CAMPINAS - SP - BRASIL.

Among mitochondrial ion channels, mitoKATP uniquely integrates metabolic cues with organelle function, yet its role in LPS tolerance has remained elusive. Using bone marrow-derived macrophages and an in vivo tolerance model, we show that downregulation of MITOK—the pore-forming mitoKATP subunit—is sufficient to enforce LPS tolerance via a ROS-mediated HIF-1 α /NF- κ B axis. Loss of MITOK inhibits Parkin-dependent mitophagy, elevates ROS, and diminishes mitochondrial adaptability, while concomitantly driving DRP1-dependent hyperfission and increased mitochondria-ER contacts. Critically, MITOK deficiency induces broad downregulation of electron transport chain complex proteins under both basal and stimulated conditions, indicating chronic mitochondrial dysfunction, yet preserves mitochondrial K⁺ homeostasis. Tolerance proceeds independently of NRF2 signaling, as demonstrated by reduced respiration and blunted TNF- α /IL-6 secretion across NRF2 knockout and control macrophages. The combined effects of impaired mitophagy, excess ROS, hyperfission with enhanced ER contacts, and reduced respiratory chain capacity collectively blunt pro-inflammatory cytokine production and compromise phagocytic capacity. By uncovering this ROS-HIF-1 α -MITOK-DRP1 circuit and its NRF2 independence, our work positions mitoKATP mitochondrial dynamics and quality control as novel targets for immunometabolic therapy in sepsis and chronic inflammation.

Keywords: MitoKATP;LPS tolerance;Mitochondria.

2869 - Category: IMMUNOREGULATION (IR)

Rho-kinase as a central regulator of hepatic insulin resistance, senescence, and inflammation during aging

MUÑOZ, V.R.¹; GASPAR, R.C.²; BURGER, B.³; NAKANDAKARI, S.C.B.R.⁴; PITOLI, B.D.S.²; DIAS, L.M.²; DE ASSIS, H.C.⁵; RODRIGUES, H.G.³; VINOLO, M.A.R.⁵; ROPELLE, E.R.²; CINTRA, D.E.⁴; DA SILVA, A.S.R.¹; PAULI, J.R.². 1. POSTGRADUATE PROGRAM IN PHYSICAL EDUCATION AND SPORT, SCHOOL OF PHYSICAL EDUCATION AND SPORT OF RIBEIRÃO PRETO, UNIVERSITY OF SÃO PAULO (USP), RIBEIRÃO PRETO - SP - BRASIL; 2. LABORATORY OF MOLECULAR BIOLOGY OF EXERCISE (LABMEX), UNIVERSITY OF CAMPINAS (UNICAMP), LIMEIRA - SP - BRASIL; 3. LABORATORY OF NUTRIENTS AND TISSUE REPAIR, SCHOOL OF APPLIED SCIENCES, UNICAMP, LIMEIRA - SP - BRASIL; 4. LABORATORY OF NUTRITIONAL GENOMICS (LABGEN), UNIVERSITY OF CAMPINAS (UNICAMP), LIMEIRA - SP - BRASIL; 5. LABORATORY OF IMMUNOINFLAMMATION, DEPARTMENT OF GENETICS, EVOLUTION, MICROBIOLOGY, AND IMMUNOLOGY, INSTITUTE OF BIOLOGY, UNIVERSITY OF CAMPINAS, CAMPINAS - SP - BRASIL.

Aging is a natural condition associated with impaired insulin action, hepatic diseases, and type 2 diabetes (T2D). However, the molecular mechanisms linking selective insulin resistance to hepatic inflammation and cell senescence remain unclear. Rho-kinase (ROCK) has emerged as a key cytoplasmatic kinase implicated in insulin action, inflammation, and whole-body metabolism. Here, we found that ROCK is hyperactivated in the liver of aged C57Bl/6J mice, contributing to insulin resistance, hepatic steatosis, inflammatory state, and increased gluconeogenesis. Both exercise (7 days, treadmill) and ROCK inhibition (7 days, Y-27632, 30 mg/kg) reversed these alterations by reducing ROCK activity and restoring hepatic insulin sensitivity and whole-body glucose homeostasis. In HepG2 cells and primary hepatocytes, genetic manipulation of ROCK1/2 showed that both ROCK1 and ROCK2 mediates insulin resistance (impaired pIR^{Y1150/1151}, pIRS1^{Y612}, pAkt^{S473}, pGSK3^{S9}) promoting insulin-stimulated mTOR phosphorylation, and upregulating lipogenesis (Srebp1c, Acc, Fas, Scd1), gluconeogenesis (G6pc, Pck1, Pcb), senescence (P21, P16, P53), and inflammation (Nfkb, Tnfa, Ikk, Il1b, Ptp1b). Mechanistically, the ability of exercise to reduce hepatic ROCK activity was lost after antibiotic (Abx) treatment, suggesting gut microbiome involvement. The microbiota-derived metabolite butyrate reduced ROCK activity in vitro and in livers of tributyrin-treated mice. Moreover, butyrate receptor (GPR43) knockout led to aberrant hepatic ROCK activation (~2-fold), while a GPR43 agonist reduced ROCK activity by ~60% in hepatocytes. These findings indicate that butyrate acts through GPR43 to suppress ROCK. Thus, we describe a gut-liver axis in aging, where GPR43-mediated ROCK activation impairs hepatic metabolism. In contrast, exercise or ROCK inhibition restore hepatic insulin sensitivity, supporting ROCK as a key mediator of microbiome-driven hepatic dysfunction and aging-related insulin resistance.

Keywords: Insulin Resistance;Rho-kinase;Aging.

2882 - Category: IMMUNOREGULATION (IR)

Macrophage AHR Deficiency Drives Obesity-Associated Metabolic Dysfunction

CASTELUCCI, B.G.¹; DE CASTRO, G.²; DA SILVA, J.V.V.¹; SCHOENFELT, K.³; MARTINS, A.J.E.¹; DA SILVA, G.R.¹; GOLDSTEIN, G.N.R.⁴; BERÇOT, M.R.¹; MACÊDO, P.E.F.⁴; DOS REIS, L.M.¹; SANTOS, W.G.S.¹; DAVANZO, G.G.¹; KESKEY, R.⁵; MELTZER, R.³; GUADAGNINI, D.¹; SAAD, M.J.A.¹; NAKAYA, H.I.⁴; CONSONNI, S.R.¹; BECKER, L.³; VIEIRA, P.M.M.D.M.¹. 1. UNICAMP, CAMPINAS - SP - BRASIL; 2. USP, CAMPINAS - SP - BRASIL; 3. UNIVERSITY OF CHICAGO, CHICAGO - ESTADOS UNIDOS DA AMERICA; 4. USP, SÃO PAULO - SP - BRASIL; 5. UNIVERSITY OF CAMPINAS, CHICAGO - ESTADOS UNIDOS DA AMERICA.

The *aryl hydrocarbon receptor* (AHR) is one of the central regulators of immune cell fitness, directly impacting macrophage (MØ) profile. Once activated, AHR modulates distinct toll-like receptor signaling cascades to adjust MØ activity in response to endogenous and exogenous ligands. Studies with AHR-*null* mice suggest that AHR signaling affects obesity development. Whether AHR signaling in MØ contributes to obesity-induced insulin resistance and chronic inflammation is still unknown. Herein, we aimed to determine how AHR in MØ contributes to obesity-induced insulin resistance and systemic inflammation. We show that AHR is downregulated in MØ under obesogenic stimulation, increasing TNFa expression. Next, we fed *Ahrfl/flLysMcre+* (KO) and *Ahrfl/flLysMcre-* (FL) mice with a chow (CD) or a high-fat diet (HFD) for 15 weeks. HFD-fed MØ KO mice presented increased weight gain, fat mass and glucose intolerance compared to FL control. Liver, muscle, and epididymal adipose tissue (epiWAT) of MØ KO mice fed a HFD showed decreased AKT phosphorylation upon insulin treatment. HFD-fed MØ KO mice presented higher liver lipid-depots as well as epiWAT and liver inflammation. *In vitro*, palmitate (palm) treatment boosted the activation of p65, p38 and JNK pathways and increased the secretion of TNFa in KO MØ. Co-treatment of FL MØ with Ahr agonist (FICZ) and palm decreased TNFa expression and secretion compared with palm alone, indicating that AHR is necessary for MØ fitness in obesity. Treatment of FL metabolic activated MØ (Mmes) with FICZ increases RAC1 phosphorylation, reducing JNK and P38 activation and decreasing TNFa secretion. FICZ treatment also inhibited the detrimental effect of Mmes over hepatocyte insulin sensibility without affecting MØ bactericidal activity *in vitro*. These findings indicate that loss of Ahr signaling in MØ exposed to an obesogenic environment alters MØ inflammatory tonus, directly impacting obesity-induced inflammation and insulin resistance.

Keywords: Ahr;macrophages; TNFa, obesity, mice.

2888 - Category: IMMUNOREGULATION (IR)

B-lymphocyte-intrinsic MyD88 signaling controls the intestinal TREG-IgA-microbiota axis under the steady-state

ALVES, I.N.D.S.¹; PACINI, C.P.¹; VIDAL, V.M.¹; FERREIRA, L.M.¹; MARCONDES, Y.L.²; DE LIMA, L.D.C.²; MASSET, M.E.G.¹; GARCIA, D.D.A.¹; SANTOS, C.S.P.D.O.²; DOS SANTOS, H.F.²; MONTEIRO, A.C.²; NÓBREGA, A.¹; BELLIO, M.¹; CANTO, F.². 1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRASIL.

Peripheral changes in CD4⁺Foxp3⁺ regulatory T (Treg) cells homeostasis are linked to inflammatory or immunosuppressive conditions, highlighting the need to understand mechanisms sustaining them. B lymphocytes help maintain Treg levels in peripheral lymphoid organs, but their role in gut-associated Treg homeostasis is unclear. Upon sensing microbial components via Toll-like receptors (TLRs) and MyD88-dependent pathways, B cells produce interleukin-6 (IL-6), which inhibits Treg differentiation in intestinal lymphoid tissues. The large intestine harbors the largest and most diverse Treg pool, whose homeostasis is shaped by commensal microbiota composition. Immunoglobulin A (IgA), mainly produced in Peyer's patches (PPs), modulates the microbiota and preserves mucosal balance. Treg cells influence IgA repertoires, which foster commensal diversity and promote intestinal Treg expansion, establishing a Treg-IgA-microbiota feedback loop. This study investigates how B cell-intrinsic MyD88 signaling regulates colonic Treg dynamics and sustains this axis. Colonic Treg cell levels were compared by flow cytometry between *Cd19^{Cre}Myd88^{fl/fl}* (B-cell-specific MyD88 deficiency) and *Myd88^{fl/fl}* mice. Treg frequencies were significantly increased in *Cd19^{Cre}Myd88^{fl/fl}* colonic lamina propria. Spleen and mesenteric lymph node leukocytes from these mice showed reduced IL-6⁺ B cells after LPS stimulation in vitro, and colonic explants secreted less IL-6. Flow cytometry revealed fewer IgA⁺ B cells in PPs and reduced fecal IgA-coated bacteria in *Cd19^{Cre}Myd88^{fl/fl}* mice. 16S rRNA gene sequencing (V4-V5) showed segregation from *Myd88^{fl/fl}* donors never co-housed with *Cd19^{Cre}Myd88^{fl/fl}*. We propose that B cell-intrinsic MyD88 signaling sustains IL-6 production in the colon, limiting local Treg differentiation. Its effects on PPs preserve IgA⁺ B cells and IgA-coated bacteria, shaping microbiota composition and a colonic environment permissive for IL-6, thus regulating the Treg-IgA-microbiota axis.

Keywords: regulatory t cells;b cells;IL-6.

2902 - Category: IMMUNOREGULATION (IR)

Rapamycin selectively modulates B-1 lymphocyte function and points to feedback regulation of the PI3K/mTOR pathway

PINHEIRO, C.O.D.D.¹; PEREIRA, M.R.¹; NEVES, C.L.²; KATO, E.E.²; VESSONI, S.C.S.²; CURI, R.³; ALVARES-SARAIVA, A.M.¹. 1. UNIVERSIDADE PAULISTA, SÃO PAULO - SP - BRASIL; 2. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE CRUZEIRO DO SUL, SÃO PAULO - SP - BRASIL.

Cellular function relies on metabolic pathways to perform vital processes, such as the PI3K/mTOR pathway, which plays a key role in immune cell activity, contributing to both innate and adaptive immune responses. Little is known about the involvement of the mTOR pathway in the biology of B-1 lymphocytes, a distinct immune cell population. This study aimed to characterize the PI3K/mTOR pathway in B-1 lymphocytes and investigate its influence on functional parameters. Phenotype, viability, proliferation, and enzymatic activity of B-1 lymphocytes cultured with rapamycin, a known selective inhibitor of mTORC1, were evaluated, along with the expression of genes and proteins related to the PI3K/mTOR pathway. Rapamycin treated cells exhibited increased proliferation and a reduced percentage of CD19⁺F4/80⁺ cells, a phenotype associated with differentiation into B-1-derived phagocytes. No significant changes were observed in viability, morphology, glucose uptake, or enzymatic activity, although gene expression revealed decreased transcripts related to glucose and fatty acid metabolism. Cells cultured with rapamycin showed increased protein levels of mTOR and phosphorylated mTOR (S2448 and S2481), as well as Raptor, but not GβL. Rictor expression was absent in both conditions. Gene expression analysis of purified peritoneal B-1 lymphocytes confirmed expression of mTOR, Akt3, Mlst8 (GβL), Raptor, and PI3K, but not Rictor. Pyruvate kinase activity was higher than other glycolytic enzymes under basal conditions. Altogether, rapamycin induced selective effects in B-1 cells, affecting gene expression and differentiation without impairing core functions. These findings highlight the complexity of mTOR regulation in B-1 lymphocytes and support future studies on their metabolic and immunological control.

Keywords: B-1 lymphocytes;PI3K/mTOR signaling;Rapamycin.

2923 - Category: IMMUNOREGULATION (IR)

Immunometabolic Reprogramming of Neutrophils in Sepsis: A Proteomic and Functional Perspective

SOUSA, M.B.; LEITE, G.G.F.; HAYASHI, J.Y.; TASHIMA, A.K.; SALOMÃO, R.. UNIFESP, SÃO PAULO - SP - BRASIL.

Introduction: "In sepsis, the immune response is driven by leukocytes, with neutrophils as the first defense. During activation, energy metabolism sustains function, mainly through glycolysis. We analyzed proteomic and functional changes in polymorphonuclear neutrophils (PMNs) from septic patients versus healthy volunteers.

Methods: We enrolled 45 septic patients and 10 healthy volunteers as controls. PMN (polymorphonuclear cell) samples were collected for processing. Proteins were trypsin-digested and labeled using Tandem Mass Tag. Data were processed with Proteome Discoverer Suite and analyzed in R. Real-time metabolic analysis of extracellular acidification rate and oxygen consumption rate was performed, along with a functional assay evaluating metabolic modulation of phagocytic capacity.

Results: Of the 1,168 proteins identified, 813 were present in over 50% of samples. Among these, 333 proteins were differentially abundant in sepsis versus controls, with 281 upregulated and 52 downregulated. Untargeted Proteomic Set Enrichment Analysis revealed profound metabolic reprogramming in septic neutrophils. Compared to healthy controls, septic PMNs showed significantly elevated scores for mitochondrial function, glycolysis, mTORC1 signaling, hypoxia response, and fatty acid β -oxidation (all $p < 0.001$), indicating a coordinated upregulation of oxidative and glycolytic pathways. We also observed enhanced glycolytic activity and respiratory capacity in patient cells, highlighting marked metabolic reprogramming. In exploring how dysregulated metabolism impairs immune function in sepsis, we found that inhibition of glycolysis and the pentose phosphate pathway (PPP) significantly reduced neutrophil phagocytic capacity.

Conclusion: Analyses reveal major metabolic alterations in septic patients, with increased activity in glycolysis and the PPP, along with, respiratory pathways, that support immune function.

Keywords: Neutrophil;Proteomic;Immunometabolism.

3408 - Category: IMMUNOREGULATION (IR)

The adenosinergic signaling regulates the severity of sepsis and the neutrophil extracellular traps release

PAMPOLHA, A.F.O.¹; RAMOS, A.D.S.¹; LEANDRO, M.D.O.¹; COSTA, V.F.¹; AUGUSTO, P.S.D.A.¹; SIZO, F.D.J.P.D.F.²; DA SILVA, M.C.F.²; ROMÃO, P.R.T.³; CUNHA, F.Q.¹; MONTEIRO, M.C.². 1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. UNIVERSIDADE FEDERAL DO PARÁ, BELÉM - PA - BRASIL; 3. UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL.

Neutrophils express different purinergic receptors, including four G protein-coupled membrane receptors (A1, A2A, A2B, and A3) that regulate primary functions of these cells, such as migration and production of inflammatory mediators, including neutrophil extracellular traps (NETs). In sepsis, NETs have microbicidal properties; however, when in excess, they contribute to organ damage and consequent multiple organ failure, worsening the clinical condition. In this study, we investigated the role of adenosine in NET release and in the progression of experimental sepsis using the cecal ligation and puncture (CLP) and endotoxemia models. We observed that treatment of mice subjected to both models with adenosine deaminase (ADA), an enzyme that metabolizes adenosine into inosine, aggravated organ damage and reduced the survival rate of septic and endotoxemic animals. Based on these findings, we demonstrated that in vitro NET production by PMA-stimulated neutrophils was increased by ADA treatment and reduced by NECA, a non-selective agonist that mimics adenosine. The modulation of NET production by adenosine was attributed to the activation of A2AAR receptors, as evidenced by in vitro and survival assays in mice with conditional deletion of the A2A receptor in neutrophils (A2AAR^{fl/fl};LysM-Cre). In conclusion, our results suggest that, during sepsis, adenosine is released and acts by reducing NET production through activation of the A2AAR receptor.

Keywords: Adenosine;Neutrophils, Neutrophil Extracellular Traps (NETs);Sepsis.

3416 - Category: IMMUNOREGULATION (IR)

Effect of *Aedes aegypti* salivary components on experimental sepsis: neutrophil modulation

BERNARDI, J.D.M.¹; DE MATOS, C.M.²; MORETTI, E.H.¹; STEINER, A.A.¹; NUNES, A.D.S.¹. 1. UNIVERSIDADE DE SÃO PAULO - USP, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO - USP, SÃO PAULO - SP - BRASIL.

Aedes aegypti are vectors of arboviruses such as dengue, yellow fever, and Zika. Transmission occurs during the blood meal and depends on overcoming the vertebrate host's hemostatic and immune defenses. To feed successfully, the mosquito secretes saliva containing molecules with anti-hemostatic, immunomodulatory, and anti-inflammatory properties. These bioactive compounds, identified in saliva and salivary gland extract (SGE), have been tested as immunobiologics in models of inflammatory and autoimmune diseases. Given their immunomodulatory potential, we propose exploring their use in sepsis, a condition in which there is an unbalanced functioning of the immune system. Thus, to evaluate the activity of SGE in sepsis *in vivo*, we used two experimental models: I) LPS-induced endotoxemia; and II) polymicrobial sepsis induced by cecal ligation and perforation (CLP). In addition, we evaluated the function of murine and human neutrophils in this context *in vitro*. Our results showed that intraperitoneal treatment with SGE after induction of endotoxemia increased survival and reduced lesion areas in the lungs of the animals, together with an increase in IL-10 in this organ. In the CLP model, treatment with SGE did not alter the production of cytokines in the organs and serum of the animals, nor did it alter mortality or the percentage of lung tissue damage assessed. *In vitro*, we observed a decreased expression of CD11b and an increase in CD66a by murine neutrophils preincubated with SGE and stimulated with PMA. Lastly, SGE was able to reduce the production of ROS and reduce the expression of CXCR2 in human neutrophils upon PMA stimulation. These data demonstrate a regulatory response pattern with a selective decrease of inflammatory parameters and cell migration, that could be beneficial in cases of endotoxemia, reducing tissue damage. New experiments should be carried out in order to obtain more robust results on the effect of SGE in a polymicrobial sepsis model.

Keywords: Sepsis;neutrophils;*Aedes aegypti*.

2691 - Category: INNATE IMMUNITY (IN)

Impaired CD1c+ Dendritic Cell Responses and Lipid Metabolism Dysregulation in Severe COVID-19

FERREIRA, G.M.¹; RIBEIRO, Á.L.¹; DE PONTES, L.G.¹; AZEVEDO, P.N.²; DA MATA, C.P.S.M.¹; CLARINDO, F.A.¹; SABINO, A.D.P.¹; DA FONSECA, F.G.¹; BEHESHTI, A.³; SORGI, C.A.². 1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 2. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. SPACE BIOSCIENCES DIVISION, NASA AMES RESEARCH CENTER, CALIFORNIA - ESTADOS UNIDOS DA AMERICA.

In December 2019, a pneumonia outbreak caused by SARS-CoV-2 emerged in China. While progress has been made, key aspects of the immune response, particularly the role of dendritic cells (DCs), remain unclear. DCs are essential in antiviral defense, working alongside macrophages as initial barriers. Among conventional DCs, CD1c+DCs exhibit high versatility, yet the CD1c-mediated lipid antigen presentation pathway is poorly understood. This study investigated CD1c+DC profiles in severe COVID-19. Using samples from SARS-CoV-2 PCR-positive patients, we applied a multi-omics approach—total-RNA-seq, flow cytometry, and lipidomics—to assess CD1c+DC functionality. RNA-seq revealed altered antigen-presenting cell (APC) gene expression across tissues, with reduced immune activity in lymph nodes but heightened responses at the primary sites of infection. CD1c downregulation correlated with poor outcomes. Flow cytometry demonstrated DC dysfunction in severe cases, characterized by impaired activation and maturation, including fewer naive CD1c+DCs and elevated mature CD1c+DCs in fatal cases. *In vitro* stimulation with inactivated SARS-CoV-2 confirmed these findings, showing reduced DC maturation and lower CD1c+ expression. Lipidomics identified dysregulated lipid profiles in severe COVID-19, with increased glycerophospholipids and lysophospholipids. Correlation network analysis revealed outcome-dependent differences: discharged patients had minimal lipid-inflammatory mediator interactions, whereas fatal cases exhibited strong associations between lipid clusters, DCs, and inflammatory mediators such as CCL2, IFN-γ and IL-18. These results suggest lipid metabolism disturbances may contribute to DC dysfunction and disease severity. Although the exact role of endogenous lipids in CD1c-mediated antigen presentation remains uncertain, our study identifies potential lipid candidates for further *in vitro* validation, which may clarify their impact on CD1c in COVID-19 pathogenesis.

Keywords: COVID-19;immune response;dendritic cell.

2783 - Category: INNATE IMMUNITY (IN)

PPAR γ -mediated effects of oleic acid on macrophages highlight a potential therapeutic target in psoriasis

BURGER, B.¹; SAGIORATO, R.N.¹; BACCAIN, I.B.¹; CERNA, G.²; ANDREEV, I.²; HALL, N.V.P.A.³; SEREZANI, H.⁴; VINOLO, M.A.R.³; SILVA, H.M.²; RODRIGUES, H.G.¹. 1. UNICAMP, LIMEIRA - SP - BRASIL; 2. RAGON INSTITUTE OF MASS GENERAL, MIT, AND HARVARD, CAMBRIDGE - ESTADOS UNIDOS DA AMERICA; 3. UNICAMP, CAMPINAS - SP - BRASIL; 4. VANDERBILT UNIVERSITY MEDICAL CENTER, NASHVILLE - ESTADOS UNIDOS DA AMERICA.

Immune dysregulation in skin can trigger chronic conditions like psoriasis. Omega-9 synthesis pathway is reduced in individuals with psoriasis and in the imiquimod (IMQ)-induced psoriasis mouse model, which also presents decreased serum oleic acid (OA) levels. Our aim was to investigate the effects of oral OA supplementation during psoriasis. Male C57BL/6 mice (n=5–9/group) were divided into 3 groups: (C) supplemented with water; (IMQ) treated with 60 mg of 5% IMQ cream and water-supplemented; and (IMQ+OA) treated with IMQ and orally supplemented with 50 μ L of OA. Skin thickness was assessed daily and skin samples were collected for histology, molecular analyses, and immunophenotyping. In vitro, dermal macrophages were treated with OA (300 μ M) and IMQ (30 μ g/mL), and cell death was assessed via propidium iodide (PI) incorporation. OA supplementation attenuated IMQ-induced skin thickening and *S100a8/a9* gene expression. Flow cytometry showed that OA restored macrophage populations reduced by IMQ. BrdU labelling revealed increased macrophage proliferation in IMQ, maintained at baseline levels with OA. Dextran-based tracking showed decreased macrophage retention in IMQ-treated mice (40% dextran⁺) vs. control (80%); the OA-treated group-maintained levels similar to controls, suggesting OA protects these cells from death. RNAseq of dermal macrophages showed that OA downregulated genes linked to apoptosis, inflammation and reactive oxygen species (ROS) process. In skin, OA reversed IMQ-induced upregulation of *Il-1 β* , *Nlrp3*, *Nr4a1*, and Caspase-1. OA also enhanced antioxidant genes *Sod2* and *Ucp2*. In vitro, OA reduced IMQ-induced IL-1 β and ROS production and protected macrophages from cell death. Pharmacological inhibition of PPAR γ (GW9662) abolished OA's protective effects in vivo, suggesting OA acts via PPAR γ activation to maintain macrophages in the tissue and attenuate inflammation. These findings highlight OA as a potential immunomodulatory therapy for psoriasis.

Keywords: Psoriasis;Fatty acids;Resident macrophages.

2920 - Category: INNATE IMMUNITY (IN)

DRP1 is necessary for macrophage IL-6 production and BAT adaptation during cold exposure.

BERÇOT, M.R.¹; DOS REIS, L.M.¹; BATAGLIOLI, B.G.C.¹; DA SILVA, J.V.V.¹; DA SILVA, G.R.¹; MACÊDO, P.E.F.²; DE VASCONCELOS, A.G.P.³; BRUNETTA, H.⁴; MORI, M.A.D.S.¹; VIEIRA, P.M.M.D.M.¹. 1. UNICAMP, CAMPINAS - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. UNIVERSITY OF WASHINGTON, WASHINGTON - ESTADOS UNIDOS DA AMERICA; 4. KAROLINSKA INSTITUTET, STOCKHOLM - SUECIA.

Brown adipose tissue (BAT) produces heat through substrate oxidation, and its activation is linked to metabolic improvement both in mice and humans. While the role of macrophages (M ϕ) in white adipose tissue (WAT) is well described, less is known about their influence on BAT thermogenic adaptation. To achieve different activation states, M ϕ heavily depend on mitochondrial fission (regulated by Drp1) and fusion (regulated by Mitofusin 1/2 (Mfn1/2)) processes. We aimed to elucidate the role of macrophage mitochondrial dynamics in BAT adaptation. Single cell RNA-seq analysis suggests that macrophages acquire a resolution-repair phenotypic activation during BAT adaptation. In vitro, DRP1^{-/-} M ϕ showed decreased frequency of repair-like M ϕ (CD206+CD301+) and heightened inflammatory M ϕ frequency (CD11c+). Furthermore, conditional deletion of DRP1 in M ϕ is sufficient to downregulate *Klf4*, *Irf4*, *Pgc1 α* , *Nrf1* and *Pparg*, and the gene expression of *Timp1*, *Timp2*, *Mmp9*, *Tgfb* and *Vegf*, which are related to extracellular matrix (ECM) remodeling. In vivo, deletion of DRP1 in M ϕ did not alter BAT expression of *Pgc1 α* , *Tnfa*, *Vegf*, and *Tgfb*, but blunted *Il6* mRNA expression decreased UCP1 and vimentin protein levels. Vimentin is associated with adipocyte differentiation. Vimentin levels are almost blunted after cold exposure in DRP1^{-/-} M ϕ . DRP1^{-/-} M ϕ also showed heightened BAT temperature but lower tail temperature, indicating impaired heat dissipation. Overall, our data indicates that expression of DRP1 in M ϕ DRP1 is necessary for the thermogenic adaptation of BAT upon cold exposure.

Keywords: brown adipose tissue;macrophages;DRP1.

2999 - Category: INNATE IMMUNITY (IN)

Evaluation Of NAD Metabolism During Monocyte Maturation Into Macrophages And Its Influence On Cell Activation By Neutrophil Extracellular Traps (NETs)

FORMIGA JUNIOR, M.A.; GONÇALVES, B.S.; ROCHAEL, N.C.; SARAIVA, E.; BOU-HABIB, D.C.. FIOCRUZ / IOC, RIO DE JANEIRO - RJ - BRASIL.

Nicotinamide adenine dinucleotide (NAD) plays a crucial role in cellular activation, acting as an enzyme substrate or cofactor. Thus, NAD cellular levels must be tightly maintained and regulated. CD38, an interferon-stimulated gene (ISG), is the main enzyme responsible for regulating NAD levels and a key inflammatory marker, underscoring its importance for the functioning of innate immune cells. Therefore, we hypothesized that there could be a correlation between CD38 activity and monocyte differentiation into macrophages, as well as with macrophage activation by neutrophil extracellular traps (NETs). NETs are molecular structures released by neutrophils upon activation by inflammatory mediators and infectious agents, formed by chromatin associated with cytoplasmic and granule proteins. We have observed that NETs activate macrophages through the NF- κ B pathway, inducing a higher production of reactive oxygen species (ROS) and inflammatory mediators. Here, we studied the CD38 activity during monocyte maturation into macrophage, and the NAD metabolism of macrophages upon interaction with NETs. We found that CD38 activity decreased during monocyte maturation and, accordingly, the intracellular levels of NAD increased in macrophages in comparison to monocytes. We detected increased levels of STING protein in macrophages compared to monocytes, pointing to the involvement of the ISG pathway in this process, and a reduction in lactate dehydrogenase (LDH) activity during macrophage maturation, suggesting that CD38 may be involved in orchestrating cellular bioenergetics beyond cellular signaling. NADase activity was increased in macrophages treated with NETs, concomitant with an enhanced production of IL-6 and TNF- α . Our results suggest that NAD metabolism regulates macrophage maturation and macrophage response to NETs through CD38 activity. Studies are in progress to evaluate intracellular NAD content in macrophages and whether CD38 regulates macrophage response to NETs.

Keywords: NAD Metabolism;Cell Activation;NETs.

3225 - Category: INNATE IMMUNITY (IN)

Activation of NLRP3 inflammasome by Bothropstoxin-I, from *B. jararacussu* snake venom, and its impact on muscle regeneration of AIRmax and AIRmin mice.

DE CAMARGO, I.M.; GIMENES, S.N.C.; TÁVORA, B.D.C.L.F.; COLOMBINI, M.; MAILHO-FONTANA, P.L.; STAROBINAS, N.; MAURO, E.F.D.L.. BUTANTAN INSTITUTE, SÃO PAULO - SP - BRASIL.

Bothrops jararacussu snake envenomation results in intense myonecrosis and local inflammation. Antivenom is the key treatment, however, it is not able to completely neutralize local damage, requiring complementary therapies. BthTX-I (K49-PLA2) in this venom, triggers myotoxicity, but its mechanism of action remains unclear. Innate immune system, including NLRP3 inflammasome, can recognize venom components, regulating inflammation and muscle regeneration. This study investigated how BthTX-I triggers inflammation and the role of NLRP3 in muscle regeneration, using AIRmax^{PyC/C} and AIRmin^{PyC/C} mice, which are genetically selected for high or low acute inflammatory responses and NLRP3 competent, and also in AIRmin^{PyT/T} with a *Pycard* gene polymorphism. BthTX-I's myotoxicity was measured by CK levels in mice serum. Leukocyte migration and muscle damage were analyzed 24h post-toxin injection using histology and flow cytometry. IL-1 β and IL-6 secretion by macrophages stimulated with BthTX-I/LPS was also evaluated. BthTX-I induced significant CK levels in all mice strains. Histology showed leukocyte recruitment and myonecrosis after 24h. AIRmax mice had higher neutrophils and early macrophages infiltration than AIRmin^{PyC/C}. AIRmin^{PyT/T} showed no significant neutrophils and macrophages at 24h post-injection compared with control. Higher IL-1 β and IL-6 levels produced by macrophages from AIRmax were induced by BthTX-I/LPS compared with those from AIRmin^{PyC/C}. BthTX-I induced lower IL-6 and absence of IL-1 β productions by macrophages from AIRmin^{PyT/T} (2way-ANOVA, Bonferroni/Tukey analysis). Our data indicate that BthTX-I triggers inflammation acting as a second signal for NLRP3 activation and IL-1 β production in AIRmax and AIRmin^{PyC/C}. In AIRmin^{PyT/T} mice, NLRP3 inactivation reduces leukocyte infiltration in local tissue. Thus, different inflammatory profiles and NLRP3 activation affect the course of inflammation and muscle regeneration. Supp.by CNPq, CAPES, Fundação Butantan.

Keywords: AIRmax;NLRP3;BthTX-I.

3229 - Category: INNATE IMMUNITY (IN)

α -Galactosidase A deficiency primes and potentiates inflammasome activation in a cellular model of Fabry disease

DE LIMA, A.B.F.¹; DIAS, M.D.J.¹; DE SOUZA, R.Q.²; SILVA, J.M.³; BOYLE, J.P.⁴; BRYANT, C.³; BORTOLUCI, K.R.¹. 1. UNIFESP, SÃO PAULO - SP - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL; 3. UNIVERSITY OF CAMBRIDGE, CAMBRIDGE - REINO UNIDO; 4. UNIVESITY OF CAMBRIDGE, CAMBRIDGE - REINO UNIDO.

Fabry disease (FD; OMIM 301500) is an X-linked storage disorder caused by deficiency of the lysosomal hydrolase α -galactosidase A (α -gal), which leads to the intralysosomal accumulation of globotriaosylceramide (Gb3). While this primary Gb3 storage triggers secondary pathways that drive disease progression, FD has not traditionally been classified as an inflammatory disease. However, emerging evidence implicates immune activation in its pathophysiology. Here, we investigate how α -gal deficiency influences inflammasome activation in a cellular model of FD. We generated α -gal-knockout (KO) THP-1 monocytes via CRISPR-Cas9 and compared them to wild-type (WT) controls. After priming with lipopolysaccharide (LPS), cells were exposed to the canonical inflammasome agonists nigericin and PrgI. Although ASC speck formation and caspase-1 activation were comparable in KO and WT cells, IL-1 β release was significantly elevated in α -gal-deficient cells for both stimuli. We then assessed the priming (signal 1) phase of inflammasome activation: upon LPS stimulation, KO cells exhibited roughly a twofold increase in IL-1 β mRNA and pro-IL-1 β protein compared to WT; besides, IL-6 transcripts were likewise upregulated. To confirm inflammasome-dependent processing, we applied inhibitors targeting cathepsin B, NLRP3, caspase-1, and gasdermin D pore formation; all of them were able to reduce IL-1 β release in both genotypes, indicating inflammasome engagement at the signal 2 stage. Together, these findings reveal a heightened inflammasome response in α -gal-deficient monocytes, especially at signal 1 level, underscoring a novel link between lysosomal storage and inflammasome activation in FD. Further studies are warranted to dissect the mechanisms—potentially including trained immunity—that underlie these alterations.

Keywords: inflammasome;lysosomal disfunction;sterile inflammation.

3329 - Category: INNATE IMMUNITY (IN)

Extracellular vesicles carrying Tissue Factor promote neutrophil aggregation during Long COVID

VIEIRA, T.S.D.S.¹; OLIVEIRA, F.D.S.¹; WRIGHT, T.D.²; DE SIMONE, C.²; DE CARVALHO, M.V.¹; SANDIM, V.³; RAJÃO, M.⁴; SEIBLITZ, G.C.¹; MORAES, E.C.D.S.¹; GONCALVES, R.M.¹; PAREDES, B.D.⁵; LIU, T.⁶; KHAN, Z.²; GARCIA, M.R.D.O.¹; NETO, H.C.D.C.F.¹; MONTEIRO, R.Q.³; NORLING, L.²; PERRETTI, M.²; BOZZA, P.T.¹. 1. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. QUEEN MARY UNIVERSITY OF LONDON, LONDRES - REINO UNIDO; 3. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 4. INCA, RIO DE JANEIRO - RJ - BRASIL; 5. BECKMAN, RIO DE JANEIRO - RJ - BRASIL; 6. QUEEN MARY UNIVERSITY, LONDRES - REINO UNIDO.

Long COVID (LC) is characterized by persistence of symptoms after SARS-CoV-2 acute infection. Among several symptoms, thrombotic anomalies and endothelial dysfunction have been reported. Mechanisms of pathogenesis remain unclear. Neutrophil (N0) intravascular activation promote thrombo-inflammation, leading to vascular occlusion and tissue malfunction. Our group demonstrated that COVID-19 survivors have high levels of plasmatic extracellular vesicles (EVs) carrying tissue factor (TF). TF can activate coagulation and protease activated receptor type 2 (PAR-2). Here, we explored the contribution of EVs carrying TF in the process of N0 intravascular activation. Analysis of EVs secretome indicated elevation of G-CSF, VEGF and SCGF-b in LC patients (EV-LC). N0 secretome revealed higher levels of MIF, CXCL12 and CLEC11a in response to EV-LC compared to control. EV-LC promoted significant N0 aggregation compared to controls. EVs did not induce NET formation nor lytic cell death. We observed direct interaction of EVs with N0 membrane. Viral infection signals enhanced endothelial cells activation, vesiculation and surface levels of TF. Accordingly, we detected elevation of total extracellular particles and CD146⁺ EVs in the plasma of patients with acute and LC. Liposomes carrying TF (Lipo-TF), used as an EV mimetic, induced aggregation, modulated chemokine receptors and MHC-II levels in N0s independently of PAR-2. Despite of this, PAR-2 agonist induced aggregation in a dose dependent manner. Aggregates showed signs of membrane fusion and membrane projections connecting cells. Lipo-TF enhance N0 aggregation, rolling and transmigration through an endothelial monolayer under inflammatory flow conditions in vitro. Hence, we demonstrated that EVs from endothelial cells carrying TF potentially promote N0 aggregation and activation, which may contribute to thromboinflammation during long COVID. Our data help shed light into the identification of potential therapeutic targets.

Keywords: Long COVID;Neutrophil;Extracellular Vesicles.

2789 - Category: MOLECULAR IMMUNOLOGY (MI)

NFATc1 transcription factor is required to maintain a fetal-exclusive human B-cell progenitor

SOUZA, O.F.¹; KOSTERS, A.²; NAZZARI, A.²; GHOSN, E.²; POPI, A.F.¹. 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. EMORY UNIVERSITY, ATLANTA - ESTADOS UNIDOS DA AMERICA.

The molecular mechanisms that explain differences in human fetal vs adult B cell development are not clarified yet. The NFAT (Nuclear Factor of Activated T cell) family of transcription factors is broadly expressed in the immune system and regulates cell survival, proliferation, cytokine production, gene expression, apoptosis, and immune effector function. Beyond five members, NFATc1 was already demonstrated to be critical to B-cell development and function in mice: deficiency of NFATc1 led to defects in the proliferation of B cells, severe B-cell lymphopenia and deficiency in peritoneal CD5+ B-1 cells (early-life B cells).

Our group mapped human B-lymphopoiesis across the lifespan using multi-omics single-cell RNA sequencing and revealed that fetal liver contain a fetal-exclusive progenitor CD7+CD10⁻, while B-progenitors in adult bone marrow are CD7-CD10⁺. To determine if humans also require NFATc1 for B-cell development, NFATc1 expression was quantified across human gestation and throughout adulthood. NFATc1 is first expressed in the hematopoietic stem-cell stage, during the pre-pro-B stage of B-cell commitment and in all B-cell stages. In silico analysis using Cell Oracle revealed that, like mice, NFATc1 regulates B-cell development in early-life but in much less extent in adulthood. These findings were validated using CRISPR-Cas9 to knockout NFATc1 on human primary CD34⁺ progenitors from fetal and adult donors. While NFATc1^{+/+} CD7⁺ fetal progenitors survive >2 weeks in cell culture, their NFATc1^{-/-} counterpart undergo cell death. In adult bone marrow samples, the knock-out of NFATc1 did not interfere in B-cell progenitor numbers.

Thus, herein the purpose is that NFATc1 may function as a master regulator of early-life B-lymphopoiesis and play a role in human B-cell-mediated diseases in childhood, including leukemias.

Keywords: B-cell development;B-1 cells;NFATc1.

3004 - Category: MOLECULAR IMMUNOLOGY (MI)

GCN2 controls ZAKα signaling to limit inflammatory activation in macrophages

REQUIAO, R.D.¹; SILVA, L.F.L.¹; MACÊDO, P.E.F.¹; DA COSTA, W.L.G.¹; ZANI, N.C.¹; XU, T.T.²; PRADO, H.¹; BENGTSON, M.¹; MURRAY, P.J.²; VIEIRA, P.M.M.D.M.¹. 1. UNICAMP, CAMPINAS - SP - BRASIL; 2. MAX PLANCK INSTITUTE OF BIOCHEMISTRY, PLANEGG - ALEMANHA.

Translational control plays a key role in macrophage function during immune responses, yet its regulation remains incompletely understood. Here, we investigate stimulus-dependent translation dynamics in primary bone marrow-derived macrophages (BMDMs) exposed to pro- (LPS + IFN γ) and anti-inflammatory (IL-4) signals. Using puromycin incorporation, we show that global translation is dynamically modulated in response to immune stimuli: pro-inflammatory activation induces a transient increase followed by suppression, while IL-4 leads to a sustained increase. Mechanistically, eIF2 α phosphorylation is modulated under pro-inflammatory conditions, implicating the integrated stress response (ISR), whereas 4EBP phosphorylation remains unchanged. We identify GCN2 as a key regulator of this process. In GCN2-deficient BMDMs, eIF2 α phosphorylation is impaired, leading to sustained translation and exaggerated cytokine production (IL-6, TNF α , IL-1 β). Proteomic profiling confirms a hyper-inflammatory phenotype. We further show that GCN2 limits activation of the ribosomal stress sensor ZAK α , which is activated upon translational stress and ribosome collisions. ZAK α inhibition in GCN2-deficient macrophages normalizes TNF α levels, indicating that GCN2 suppresses ZAK α -driven inflammatory signaling. Our findings define a GCN2–eIF2 α –ZAK α axis that restrains macrophage inflammation by sensing translational stress and modulating protein synthesis. This pathway has therapeutic implications: in the tumor microenvironment, where macrophages adopt an immunosuppressive phenotype, GCN2 inhibition may promote anti-tumor immunity. Conversely, enhancing GCN2 activity may help control pathological inflammation in autoimmune disease or sepsis. Thus, GCN2 serves as a key translational checkpoint in macrophage activation with broad relevance to immune regulation and disease.

Keywords: Macrophage;Translation;GCN2.

3156 - Category: MOLECULAR IMMUNOLOGY (MI)

MicroRNAs-205 and -193b modulate the response of human keratinocytes and macrophages to *Leishmania braziliensis* infection.

ARAUJO, S.N.D.O.¹; BASTOS, R.P.D.S.²; SILVA, S.L.S.³; RIBEIRO, B.V.⁴; LUZ, Y.D.S.¹; DA SILVA, I.B.S.¹; BARBOSA, L.E.⁵; SANTOS, R.L.⁵; DE OLIVEIRA, E.C.²; DA SILVA, G.D.¹; MARINHO, A.I.²; DE MENEZES, J.P.B.²; BOAVENTURA, V.²; OLIVEIRA, P.R.S.⁵; BRODSKYN, C.I.²; FARIAS, L.P.⁶; TAVARES, N.M.². 1. FIOCRUZ BAHIA, SALVADOR - BA - BRASIL; 2. FIOCRUZ BAHIA/UFBA, SALVADOR - BA - BRASIL; 3. USP, RIBEIRÃO PRETO - SP - BRASIL; 4. FIOCRUZ, SALVADOR - BA - BRASIL; 5. UFBA, SALVADOR - BA - BRASIL; 6. INSTITUTO GONÇALO MONIZ - FIOCRUZ BAHIA, SALVADOR - BA - BRASIL.

Cutaneous leishmaniasis (CL), a neglected disease caused by *Leishmania braziliensis* (Lb), leads to chronic ulcers and uncontrolled inflammation, with poorly understood host cell response mechanisms. Emerging evidence indicates that microRNAs (miRNAs) are key post-transcriptional regulators during infection. We first described a distinct miRNA signature in CL, identifying miR-193b as a potential biomarker of healing time. Intriguingly, comparative transcriptomics across inflammatory skin diseases (LC, lupus, psoriasis, and leprosy) revealed miR-205 as the only commonly modulated miRNA, suggesting a broader role in skin pathology. Here, we investigate the functional role of miR-193b in macrophages and miR-205 in human keratinocytes during Lb infection. We found that miR-193b exhibits an immune-responsive expression pattern, transiently suppressed during early infection (30 min and 4 h), but upregulated at 12 h, coinciding with its leishmanicidal effect. The overexpression of miR-193b reduced parasite load, amastigote number per cell, and increased ROS production. Additionally, miR-205 was consistently reduced in both CL biopsies and Lb-exposed HaCaT cells. Functional assays demonstrated that miR-205 promoted cell migration in a wound healing model, a process impaired by Lb, but rescued upon miR-205 restoration. Mechanistically, miR-205 promoted actin remodeling and FAK activation, key drivers of cell migration. While Lb reduced keratinocyte proliferation (Ki67+), miR-205 did not reverse this effect, highlighting its specific role in migration rather than growth. Our findings suggest that miR-193b and miR-205 play distinct yet complementary responses to Lb: miR-193b potentiates leishmanicidal defense in macrophages, while miR-205 facilitates tissue repair in keratinocytes. The therapeutic potential of these miRs is currently being evaluated in vivo using Lb-infected BALB/c mice. These insights may pave the way for novel host-directed therapies against CL.

Keywords: miR-193b;miR-205;Cutaneous Leishmaniasis.

3076 - Category: NEUROIMMUNOLOGY (NI)

Infection-induced disruption of the cholinergic pathway impacts mesenteric immune tone by inducing regulatory T cell reprogramming

DA CONCEIÇÃO, L.B.¹; GONÇALVES, L.M.¹; SILVA, L.M.²; LUDWIG, R.G.³; DE ARAUJO, M.V.¹; DA SILVA, G.W.¹; RODRIGUES, G.M.B.¹; ALVES, C.B.¹; DE ABREU, A.M.¹; DE ASSIS, J.B.¹; OLIVEIRA, B.D.C.¹; COELHO, M.P.S.¹; CARDOSO, A.¹; CASTELUCCI, P.²; DE OLIVEIRA, E.E.⁴; PRADO, C.M.⁵; JÚNIOR, J.D.²; ALVES FILHO, J.C.F.⁶; KANASHIRO, A.⁷; VINOLO, M.A.R.³; DA FONSECA, D.M.¹. 1. ICB-USP/INCT, SÃO PAULO - SP - BRASIL; 2. ICB-USP, SÃO PAULO - SP - BRASIL; 3. UNICAMP, SÃO PAULO - SP - BRASIL; 4. UERJ, SÃO PAULO - SP - BRASIL; 5. UNIFESP, SÃO PAULO - SP - BRASIL; 6. FMRP-USP, SÃO PAULO - SP - BRASIL; 7. FMRP - USP, SÃO PAULO - SP - BRASIL.

The gastrointestinal tract (GI) comprises the largest lymphoid tissue in the body, as it contains more leukocytes than all the lymphoid organs combined. In addition, the GI mucosa is highly exposed to environmental antigens, therefore requiring a complex network of regulatory mechanisms to sustain tissue homeostasis. We have shown that a single episode of GI infection by *Yersinia pseudotuberculosis* (YP) in mice impacts the induction of gut canonical regulatory responses. This process, which we have named as "immunological scarring", causes irreversible inflammation in the mesentery that compromises regulatory and type 2 immunity and increases susceptibility to inflammatory diseases. Here, we studied the contribution of the cholinergic pathway to the mechanisms sustaining the immunological scarring. We found that the IL-33-dependent type 2 mesenteric immune tone was negatively regulated by ACh, as the blockage of ACh-signaling increased the infiltration of type 2 cells (such as eosinophils, M2 macrophages, Th2, and ILC2) in the mesentery in an IL-33-dependent manner. Notably, YP infection promoted ACh production by leukocytes in the mesentery accompanied by a reduction in IL-33 production by stromal cells and therefore a reduction in the local type 2 and regulatory immune tone. In light of these data, the suppression of mesenteric type 2 immunity by YP was mediated by ACh and likely involved impairment of regulatory responses as well, given that intestinal regulatory T cells from YP-infected mice showed reduced accessibility at the IL-33 (*il1rl1*) receptor locus. Considering the role of IL-33 signaling in promoting Foxp3 expression in regulatory T cells, which is essential for maintaining the suppressive function of these cells, we suggest here that the impairment of regulatory and Th2 responses during YP-induced immunological scarring is dependent on the ACh-IL-33 axis. Restoring the function of this axis might drive strategies to reverse the immunological scarring.

Keywords: ACh;Mesentery;IL-33.

3161 - Category: NEUROIMMUNOLOGY (NI)

IL-21 induces neuroinflammation and alters lipid metabolism

OYAMADA, H.A.D.A.¹; YING, Y.²; AGRAWAL, S.²; LIU, A.²; SUBRAMANIAN, V.²; BENTO, C.A.D.M.³; AGRAWAL, A.².
1. UERJ, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSITY OF CALIFORNIA IRVINE, IRVINE - ESTADOS UNIDOS DA AMERICA; 3. UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Neuroinflammation is a key component in the development and progression of neurodegenerative diseases, with several cytokines, like IL-21, being implicated in these processes. However, the effects of IL-21 on brain cells, including microglia, remain poorly understood. The aim of this work was to investigate the mechanism by which IL-21 may influence neuroinflammation. To do so, young healthy C57BL/6 mice were IV injected with IL-21, with the objective of mimicking chronic exposure, for 2 weeks with a total of 5 injections. 2 days after the last injection, mice were euthanized. On the brain of the mice, increased production of TNF- α , IL-6, IL-1 α , IL-1 β , IL-18, MCP-1 and IFN- γ were found, while also showing a lower production of IL-33. Next, using flow cytometry, the expression of microglia activation markers was analyzed, with IL-21 increasing levels of MHC-II and CD68. The neutral lipidic content in these cells, marked by BODIPY dye, was also shown to be increased, as was CD36, a lipid uptake receptor, and TREM-2, a receptor associated with cholesterol and myelin metabolism in CNS. Next, the microglia cell lineage HMC-3 was also treated with IL-21, with increased production of IL-6 being observed. Using flow cytometry, it was found that IL-21 also increased the lipid content in the cell lineage, and also the levels of CD36, ApoE, a lipid transport molecule, and HIF1- α , a transcription factor associated with lipid metabolism. Interestingly, *in vitro*, IL-21 was actually capable of reducing the levels of TREM-2. We also measured the plasmatic concentration of IL-21 in aged and young individuals, and found that IL-21 levels were increased in aged subjects. Finally, aged healthy mice were also injected with IL-21, showing increased microglia expression of MHC-II, CD36, TREM-2 and neutral lipid content when compared with the younger counterparts. In summary, our results shown can increased the neuroinflammation process while also enhancing lipid accumulation in microglia.

Keywords: Neuroinflammation;Microglia;IL-21.

3188 - Category: NEUROIMMUNOLOGY (NI)

Pulmonary allergic inflammation increases microglia activation associated with depressive behavior

ROSA, U.G.C.¹; FERREIRA, G.M.D.C.²; MARTINS, N.S.¹; GEMBRE, A.F.²; PEREIRA, A.C.M.³; SILVA, J.M.D.N.⁴; ZANGROSSI JUNIOR, H.⁵; LISBOA, S.F.⁶; BONATO, V.L.D.². 1. BASIC AND APPLIED IMMUNOLOGY PROGRAM, RIBEIRAO PRETO MEDICAL SCHOOL, UNIVERSITY OF SAO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 2. DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY, RIBEIRAO PRETO MEDICAL SCHOOL, UNIVERSITY OF SAO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 3. PHARMACEUTICAL SCIENCES PROGRAM, RIBEIRAO PRETO SCHOOL OF PHARMACEUTICAL SCIENCES, UNIVERSITY OF SAO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 4. PHARMACOLOGY PROGRAM, RIBEIRAO PRETO MEDICAL SCHOOL, UNIVERSITY OF SAO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 5. DEPARTMENT OF PHARMACOLOGY RIBEIRAO PRETO MEDICAL SCHOOL, UNIVERSITY OF SAO PAULO, RIBEIRÃO PRETO - SP - BRASIL; 6. DEPARTMENT OF BIOMOLECULAR SCIENCES, RIBEIRAO PRETO SCHOOL OF PHARMACEUTICAL SCIENCES, UNIVERSITY OF SAO PAULO, RIBEIRÃO PRETO - SP - BRASIL.

Depression episodes affected approximately 300 million people worldwide in 2019. Peripheral inflammation, such as asthma, can affect circulation and reaches the brain inducing mental disorders such as depression. Individuals with asthma have a four times higher prevalence of depression compared to non-asthmatic individuals, but it is unclear how asthma may contribute to the development of this disease. The aim of this study is to investigate the effect of allergic lung inflammation on neuroinflammation in association with the development of depression. C57BL/6 mice sensitized and challenged with ovalbumin (OVA) exhibited an increase in the permeability of blood-brain barrier (BBB), evaluated by Fitc-Dextran, compared to the control group (CT group -non-sensitized, non-challenged animals). How the cellular profile in the brain can change with increased BBB permeability, we found an increase in the frequency of activated microglia (CD45^{high}CD11b⁺Ly6C⁺CX3CR1⁺), and an influx of neutrophils and CD11c⁺ cells in the brain compared to CT group. Moreover, animals exposed to the allergen significantly increased the behavior associated with depression, assessed by latency and the number of groomings in the Splash Test and by the time of immobility in the Tail Suspension Test. These behavioral tests are associated with changes in the prefrontal cortex region, which showed a local increase of microglia, neutrophils and CD11c⁺ cells compared to the CT group. In addition, using the PHATE software which evaluates cell trajectory by similarity showed that activated microglia were derived from microglia in homeostasis in the brain, and they were not derived from peripheral monocytes. Using BALB/c animals in the OVA asthma model, we found an increase in activated microglia and behavior associated with depression by Splash Test and Tail Suspension Test. Allergic lung inflammation promotes microglial activation and depressive behavior, possibly requiring tailored therapeutic approaches

Keywords: Asthma;Microglia;Depression.

3315 - Category: NEUROIMMUNOLOGY (NI)

Neuroimmune and gastrointestinal correlates of shrimp allergy in a mouse model

ANDRADE, C.M.¹; LIMA, B.G.C.²; ROGINSKI, A.C.²; DE SOUZA, N.¹; BRODSKYN, C.I.¹; FLORSHEIM, E.B.². 1. INSTITUTO GONÇALO MONIZ (FIOCRUZ/BA), SALVADOR - BA - BRASIL; 2. 2BIODESIGN INSTITUTE, CENTER FOR HEALTH THROUGH MICROBIOMES, ARIZONA STATE UNIVERSITY, TEMPE - ESTADOS UNIDOS DA AMERICA.

Shrimp is a leading cause of food allergy and anaphylaxis. Despite its high prevalence (5 to 10%) in both children and adults, experimental models replicating these allergic reactions and their mechanisms are lacking. Here, we established a novel model of shrimp hypersensitivity by adapting the gold-standard mouse food allergy to ovalbumin. Female BALB/c mice were sensitized subcutaneously with shrimp extract (*Litopenaeus vannamei*) and alum on days 0 and 14. Control mice received alum alone. All groups were orally challenged with shrimp protein on days 21, 24, 27, 30, and 33. We then determined serum levels of total IgE, intestinal inflammation and transit time, hypothermia, and acute behaviors. We quantified behavioral outputs using the open field test and elevated plus maze 24h post oral challenges with shrimp. To identify neuronal activation in brain regions, we performed immunofluorescence using the activation marker cFOS. Upon oral exposure to shrimp, allergic sensitized mice showed 16x higher total IgE levels, compared with controls. Shrimp sensitized and challenged animals exhibited intestinal transit 42% faster than controls and mild hypothermia (1.87% lower than controls), indicating anaphylaxis. No behavioral changes were observed after several oral challenges in both groups. However, repeated oral shrimp exposure activated the area postrema, nucleus of solitary tract, parabrachial nucleus, and central amygdala in the brains of allergic mice compared to controls, areas related to aversive responses and activated during infection-induced sickness behaviors. These findings suggest that the central nervous system can detect allergic inflammation to shrimp allergens without inducing anxiety-like behavior. Yet, the development of avoidance is still possible and must be investigated in future studies. Overall, this work contributes to the neuroimmune underpinnings of food allergies and suggests that brain function and behavior might be allergen-specific. Keywords: food allergy;shrimp;anxiety-like behavior.

3502 - Category: NEUROIMMUNOLOGY (NI)

Distribution and phenotypic changes of B cells in the mouse brain from development to aging

DANTAS, D.P.¹; MAIA, L.C.R.¹; CAMPOS, R.M.P.¹; GALVANI, R.G.A.²; BONOMO, A.³; VALE, A.M.¹; COELHO, P.M.P.⁴. 1. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 2. IOC - FIOCRUZ/RJ, RIO DE JANEIRO - RJ - BRASIL; 3. IOC-FIOCRUZ/RJ, RIO DE JANEIRO - RJ - BRASIL; 4. RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

B cells in the central nervous system (CNS) are newly described in the meninges, where plenty of these cells can locate, migrate, mature, and have antigen presenting (Mazzitelli, 2023). Nonetheless, less is known about where B cells are located in the brain in a steady state during life. Also, remains unclear whether chemokines and cytokines expression changes in the brain, possibly modulating the presence and activities of those cells. The aim of this work is to analyze B cells populations in the brain during life, identify which chemokines and cytokines are present in the brain in a wide range of ages and describe the position of B cells in different areas of the brain.

Brains from C57BL/6 mice from 3 days of life (P3), P21, 3 months of life (3m), 18m and 24m were used. Flow cytometry was used to analyze populations of B cells and their interaction with blood vessels. Chemokines and cytokines multiplex assay were performed. Confocal microscopy was performed to analyze the location of B cells. All experiments were approved by CEUA-UFRJ (protocol numbers: 080/17, 43/20).

We found that CD19+B cells increase in the brain with age increase. However, not all B cells CD19+ express B220. In age rise, the expression of B220+ B cells in the brain decreases. Furthermore, immature B cells decrease, and Mature B cells increase in young adult brain (3m) compared with other ages. B-1 and B-2 B cells increase from neonatal to young adult mice brain, however these cells do not increase in the aging. Notably, TNF- α , CXCL13 and CCL5 increase with the aging rise. IgM+ and/or IgD+ expressions seem to be important features to B cells being found near to blood vessels.

B cells presence in the brain is dynamic. Mature B cells increase with age as well B-1 and B-2 B cells. CXCL13 expression fit similarly to immature/transitional B cells. IgM and/or IgD expressing B cells are associated with blood vessels.

Keywords: B cells;chemokines and cytokines;brain.

2833 - Category: TRANSPLANTATION AND IMMUNOGENETICS (TR)

Rare Genetic Variants of NLRP12 in Admixed Latino-American Children With SARS-CoV-2-Related Multisystem Inflammatory Syndrome

PEDRO, R.B.D.S.¹; BARRETO, T.M.M.²; SOUZA, R.S.³; PAIVA, I.M.⁴; SILVA, A.S.⁵; NOGUEIRA, A.L.⁵; BELLINAT, A.P.⁶; DIAS, N.L.S.⁶; ARAUJO, S.N.D.O.⁷; BRITTO, G.D.S.G.¹; AMARAL, E.H.B.¹; ROCHA, G.D.⁸; CARVALHO, C.S.⁹; LYRA, R.⁹; KEHDY, F.S.G.¹⁰; CAMPOS, T.L.³; DE MOURA, P.M.M.F.⁸; SANTOS, E.T.⁹; CUNHA, T.M.⁴; TAVARES, N.M.⁷; SÁ, M.V.B.O.⁵; RAMOS, R.C.F.⁵; DO CARMO, R.F.⁸; VASCONCELOS, L.R.S.³; OLIVEIRA, P.R.S.¹. 1. INSTITUTO DE BIOLOGIA, UNIVERSIDADE FEDERAL DA BAHIA, SALVADOR - BA - BRASIL; 2. INSTITUTO COUTO MAIA, SALVADOR, SALVADOR - BA - BRASIL; 3. INSTITUTO AGGEU MAGALHÃES, FUNDAÇÃO OSWALDO CRUZ, RECIFE - PE - BRASIL; 4. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRASIL; 5. DEPARTAMENTO DE INFECTOLOGIA PEDIÁTRICA, HOSPITAL UNIVERSITÁRIO OSWALDO CRUZ, RECIFE - PE - BRASIL; 6. UTI PEDIÁTRICA, HOSPITAL MARTAGÃO GESTEIRA, SALVADOR - BA - BRASIL; 7. INSTITUTO GONÇALO MONIZ, FUNDAÇÃO OSWALDO CRUZ, SALVADOR - BA - BRASIL; 8. INSTITUTO DE CIÊNCIAS BIOLÓGICAS, UNIVERSIDADE DE PERNAMBUCO, RECIFE - PE - BRASIL; 9. INSTITUTO DE CIÊNCIAS BIOLÓGICAS, UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL; 10. INSTITUTO OSWALDO CRUZ, FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

Multisystem inflammatory syndrome in children (MIS-C) is a rare, potentially fatal complication of SARS-CoV-2 infection, characterized by systemic inflammation and multiple organ involvement. Genetic defects in inflammation-related pathways have been linked to MIS-C, but additional research is needed, especially in diverse ethnic groups. The present study aimed to identify genetic variants underlying MIS-C in Brazilian patients. Whole exome sequencing was performed, focusing on genes involved in the host immune response to SARS-CoV-2. Our findings emphasize the role of immunogenetic variation in modulating host susceptibility to MIS-C. Functional assays assessed the impact of selected variants on nuclear factor- κ B signaling. Nine rare, potentially deleterious variants were found in 8 of 21 patients, located in the IL17RC, IFNA10, or NLRP12 genes. Unlike the wild type NLRP12 protein, which inhibits nuclear factor- κ B activation in HEK 293T cells, the mutant NLRP12 proteins have significantly reduced inhibitory properties. This loss of function may contribute to the exaggerated inflammatory response observed in MIS-C. In conclusion, our results indicate that rare autosomal variants in immune-related genes may underlie MIS-C pathogenesis, highlighting the potential role of NLRP12 in its predisposition. These findings not only advance the understanding of MIS-C genetics but may also guide personalized therapeutic strategies targeting specific immune pathways.

Keywords: SARS-CoV-2; MIS-C; NLRP12.

3025 - Category: TRANSPLANTATION AND IMMUNOGENETICS (TR)

ASSOCIATION STUDY OF IMMUNOGENETIC FACTORS WITH ANXIETY AND DEPRESSION SYMPTOMS IN BRAZILIAN CHILDREN

LESSA, A.C.M.; DALTRO, J.D.P.; SANTOS, A.O.M.; BRITTO, G.D.S.G.; AMARAL, E.H.B.; PEDRO, R.B.D.S.; MARINHO, A.I.; DE JESUS, T.S.; NOVAES, S.D.; DE SOUZA, A.C.L.; DE SOUSA, E.S.C.; OLIVEIRA, P.R.S.. FEDERAL UNIVERSITY OF BAHIA, SALVADOR - BA - BRASIL.

Behavioral disorders in children are associated with both environmental and genetic factors, including those related to immune function. This study investigated the association between immunogenetic variants and symptoms of anxiety/depression (SAD) in 1,190 children from Salvador, Brazil. SAD were assessed using the Child Behavior Checklist (CBCL), with 13% of participants showing high scores (≥ 70). After genotyping (Illumina HumanOmni 2.5-8v1), imputation, and quality control, approximately 14.5 million SNPs were analyzed. Variants in *IL10* (rs3024505, $p = 5 \times 10^{-3}$; $\beta = -1.93$; rs3024495, $p = 4 \times 10^{-3}$; $\beta = -1.93$) were associated with a reduced risk of SAD. In contrast, variants in *TNF* (rs3093668 and rs3093661, $p = 7 \times 10^{-4}$; $\beta = +3.4$) and *TLR4* (rs1927903, rs0739499, rs1999066, rs1999065; $p < 0.04$) were associated with increased SAD scores. Several of these SNPs showed eQTL (expression Quantitative Trait Locus) signals in immune and brain cells/tissues. For example, rs3024505 was associated with altered expression of *CR2* and *SLC41A1* in T cells and monocytes, as well as *RAB29* and *CD46* in brain tissue, including amygdala. The variant rs3093668 was linked to differential expression of genes such as *IER3* and *TNXB* in the central nervous system, including the frontal cortex, cerebellum, and hippocampus, and in immune cells such as lymphocytes, neutrophils and macrophages. SNPs in *TLR4* also showed eQTL signals for *TLR4* expression, particularly in blood and pituitary tissue. Moreover, multiple associations were found with lncRNA expression in brain regions including the hippocampus, frontal cortex and basal ganglia. Collectively, these findings suggest that immunogenetic variants may contribute to the development of SAD in childhood, potentially mediating the impact of social and environmental factors through dysregulation of neuroimmune pathways. These results highlight immunogenetics as a critical interface linking inflammation, neurodevelopment, and child mental health.

Keywords: Immunogenetics; anxiety ;Children.

2762 - Category: TUMOR IMMUNOLOGY (TU)

Evaluation of the effect of the co-culture with dendritic cells in the phenotype and function of iPSC-derived CAR-T cells

CATHARINO, L.C.C.¹; RIBEIRO, P.D.A.C.¹; OLIVEIRA, T.G.M.²; ROCHA, V.G.²; RAMOS, R.N.³; DALEY, G.Q.⁴; BARBUTO, J.A.M.⁵. 1. ICB-USP/HCFMUSP, SÃO PAULO - SP - BRASIL; 2. HCFMUSP, SÃO PAULO - SP - BRASIL; 3. IDOR/HCFMUSP, SÃO PAULO - SP - BRASIL; 4. HARVARD UNIVERSITY, BOSTON - ESTADOS UNIDOS DA AMERICA; 5. ICB-USP, SÃO PAULO - SP - BRASIL.

Given the manufacturing difficulties still encountered in autologous CAR-T cell therapies, off-the shelf alternatives are being studied and developed to help overcome these hurdles. In this scenario, the use of human iPSC (Induced Pluripotent Stem Cells) for the generation of CAR-T cells is an interesting alternative. However, most iPSC differentiation to T cells protocols listed in the literature promote the generation of only CD8⁺ T cells in feeder-free culture systems, which might restrict the long-term efficacy of the CAR-T cells obtained by this approach. Therefore, this project aims to generate CAR-T cells from iPSC using a co-culture system with monocyte-derived dendritic cells (Mo-DC), in order to enable the generation of CD4⁺ T cells from iPSC. The iPSC was initially differentiated to hematopoietic stem/progenitor-like cells. High frequencies of CD34⁺ cells were generated, and CD43 and CD45 coexpression was observed. When differentiating these cells to T lymphocytes, only T CD8⁺ cells were generated in the control protocol, as expected, and these cells were mostly CD8αα⁺, TCRγδ⁺ and CD7⁺CD5⁻. In the differentiation in co-culture with Mo-DC, the generation or expansion of CD4⁺ T cells was observed by the end of differentiation, at variable frequencies, only when an activation with anti-CD3/CD28 antibody was also performed. These CD4⁺ T cells were CD5⁺CD7⁻ or CD5⁺CD7⁺ and expressed either TCRγδ or TCRαβ. The CD8⁺ or CD4⁺ T cells obtained in all experimental groups were capable of producing IFN-γ, TNF-α and TGF-β1, individually or in combination, after nonspecific stimulation. Taken together, these data demonstrate that co-culture with Mo-DC, along with activation with anti-CD3/CD28 antibody during the differentiation of iPSC to T lymphocytes, provides an increase in the mean frequency of CD4⁺ T cells generated by the end of differentiation. However, further analyses need to be performed to determine the origin of these CD4⁺ T cells.

Keywords: Induced Pluripotent Stem Cells (iPSC);CAR-T cells;Dendritic cells.

2781 - Category: TUMOR IMMUNOLOGY (TU)

Trained immunity induced by Bacillus Calmette-Guérin (BCG) enhances systemic antitumor response improving bladder cancer treatment

DE QUEIROZ, N.M.G.P.¹; BORGES, V.M.¹; MARINHO, F.V.²; DE ARAUJO, A.C.V.S.C.¹; DE SOUZA, R.P.³; OLIVEIRA, S.C.¹. 1. DEPARTAMENTO DE IMUNOLOGIA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO (USP), SÃO PAULO - SP - BRASIL; 2. DEPARTAMENTO DE BIOQUÍMICA E IMUNOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS, UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL; 3. DEPARTAMENTO DE GENÉTICA, ECOLOGIA E EVOLUÇÃO, INSTITUTO DE CIÊNCIAS BIOLÓGICAS, UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRASIL.

Bacillus Calmette-Guérin (BCG) modulates the tumor microenvironment (TME) by inducing local and systemic antitumor response. The aim of this study is to evaluate the effect of BCG-induced trained innate immunity to potentializes systemic antitumor response. To investigate the mechanisms involved in the systemic response induced by BCG immunotherapy we established a systemic mouse model comprehending the injection of MB49 tumor cells subcutaneously into both flanks of the animals (local and abscopal tumors), and only the local tumor in the right flank was treated with intratumoral (IT) BCG or PBS (mock). We demonstrated that BCG-IT treatment promotes the infiltration of inflammatory cells (neutrophils, macrophages, dendritic cells, NKT and CD8⁺ T cells) that controls both local and abscopal tumors in wild type mice. The systemic model was evaluated in several knockout mice related to adaptive immunity (Rag1^{-/-}, CD4^{-/-}, CD8^{-/-}, IFNAR^{-/-} and IFNγ^{-/-}) and we identified CD8⁺ T cells and IFN-γ as essential players for tumor control. Furthermore, we analyzed the effect of immunization with intravenous (IV) BCG to induce trained immunity before starting the protocol of systemic tumor model. BCG-induced trained immunity potentializes bone marrow-derived macrophages (BMDMs) immunometabolic activation via Akt/mTOR/S6K/HIF-1α and enhances cytokine production. Also, trained immunity enhances the recruitment of immune cells into TME, IFN-γ secretion by CD8⁺ T cells and cytotoxic response, leading to a strong systemic antitumor response that controls tumor growth. Our results reinforce the potential application of BCG-induced trained immunity to induces a systemic antitumor response, crucial for cancer immunotherapy efficacy.

Keywords: Bacillus Calmette-Guérin (BCG);Bladder Cancer Immunotherapy;Trained Immunity.

2827 - Category: TUMOR IMMUNOLOGY (TU)

FOXP3+ CD4 T follicular cells potentially restrict tertiary lymphoid structure and germinal center development in endometrial cancer patients

PIERRE, M.L.M.; KINKER, G.S.; GUSMAO, A.F.; CABRAL-PICCIN, M.P.; CAMBUI, R.; ARAUJO, E.V.D.O.; ELIAS, B.D.D.S.; CARVALHO, M.L.R.; DELECHIAVE, D.; DOS SANTOS, J.P.; GONÇALVES, B.T.; DE LIMA, V.C.C.; DA COSTA, A.A.B.A.; MEDINA, T.D.S.. A.C.CAMARGO CANCER CANCER, SÃO PAULO - SP - BRASIL.

Endometrial cancer (EC) is among the most common gynecological malignancies. Despite its promise, immunotherapy benefits fewer than 50% of EC patients. The lack of complete response is, at least in part, due to immune cell exhaustion that leads to poor tumor elimination. Moreover, our and other groups have shown that immune cells organized within cell aggregates, such as tertiary lymphoid structures (TLS), elicit potent in-situ antitumor responses. Recently, evidence shows that Foxp3+ T follicular regulatory (TFR) cells negatively impact organization and function of secondary lymphoid structures. Therefore, this study investigates whether TFR cells could be operating as negative regulators of TLS development and maturation in EC, which may represent an unrecognized barrier to effective ICB immunotherapies. To this end, samples from untreated EC patients were analyzed by flow cytometry (n=21), immunohistochemistry (IHC) (n=13), and scRNA-seq (n=7). Through flow cytometry, we found that CD4 and CD8 T cells exhibited phenotypes suggestive of tumor reactivity, with increased expression of activation (CD39, CD69), tissue-residency (CD103), and exhaustion (PD-1) markers. Additionally, tumor-associated TLS was identified in most EC patients, some with germinal center (GC), indicative of mature TLS. Interestingly, higher FOXP3+ cell frequencies inside TLS were correlated positively with immature TLS, suggesting they may hinder TLS development. In addition, IHC and scRNA-seq showed increased IgG+ plasma cells in patients with mature TLS, suggesting that antigen-responsive B cells undergo cell differentiation and expansion in situ. Finally, scRNAseq results revealed that mature TLS are linked to increased T follicular helper (TFH) cells, key GC drivers, while TLS- patients displayed only T follicular regulatory (TFR) cells, known to suppress TFH cells in GCs. Overall, our results suggest FOXP3+ CD4 T cells may limit TLS maturation and GC development in EC patients.

Keywords: : Tertiary Lymphoid Structures (TLS);T follicular cells;Endometrial cancer.

2841 - Category: TUMOR IMMUNOLOGY (TU)

Epigenetic Modulation as a promising approach to enhance NK cell functional features in breast cancer patients

DELECHIAVE, D.¹; KINKER, G.S.¹; GUSMAO, A.F.¹; ARAUJO, E.V.D.O.¹; PIERRE, M.L.M.¹; ELIAS, B.D.D.S.¹; CARVALHO, M.L.R.¹; CAMBUI, R.A.G.¹; DOS SANTOS, J.P.¹; PICCIN, M.P.C.¹; DE LIMA, V.C.C.²; MEDINA, T.D.S.². 1. A C CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 2. A C CAMARGO CANCER CENTER, SAO PAULO - SP - BRASIL.

Cancer remains one of the leading causes of death worldwide. Among the current therapeutic strategies available, immunotherapy aims to restore the immune system's ability to eliminate malignant cells. Natural killer (NK) cells are key players in antitumor response, by targeting and killing tumor cells. Thus, their use in the context of adoptive cell therapy is now gaining momentum. Despite encouraging results, several limitations still hinder the clinical translation of this approach, including the immunosuppressive nature of the tumor microenvironment, which contribute to immune cell exhaustion. Epigenetic modulation has been studied to enhance the functional state of immune cells. Therefore, our aim is to analyze NK cell features in cancer context, and to investigate potential epigenetic inhibitors capable to improve their functionality. We conducted a phenotypic characterization of NK cells from the peripheral blood of treatment-naïve solid tumor patients (endometrial, gastric, renal and breast cancers) by flow cytometry. Compared to healthy donors, patient-derived NK cells exhibited increased activation, exhaustion and dysfunction-associated markers. Among them, breast cancer patients showed greater expression of TIGIT, TIM-3 and PD-1 and lower expression of granzyme B. To overcome this dysfunctional state, NK cells from breast cancer patients were treated with different epigenetic inhibitors, in order to identify the most effective compound. A-485, a selective inhibitor of the CBP/p300 complex, enhanced the expression of functional factors (Granzyme B, Perforin, IFN- γ , and TNF- α) and downregulated exhaustion markers (PD-1, TIM-3, and TIGIT) in NK cells. Overall, our findings highlight the potential of epigenetic modulation by A-485 in reversing NK cell dysfunctional features in breast cancer.

Keywords: Natural Killer Cell;Epigenetic Modulation;Breast Cancer.

2844 - Category: TUMOR IMMUNOLOGY (TU)

Engineering NK cells to optimise liver homing and block PD-L1-mediated inhibition of T cells for adoptive cell therapy of liver cancer

DINIZ, M.D.O.; ZHONG, Y.; KHAWAJA, N.; KUCYKOWICZ, S.; ROMERO, D.B.; DAVIES, J.; MCDOWELL, J.; COUNSELL, J.; MAINI, M.. UNIVERSITY COLLEGE LONDON, LONDON - REINO UNIDO.

NK-cell adoptive therapy holds promise in hepatocellular carcinoma (HCC) but is limited by inadequate tumour homing and lack of harnessing complementary T-cell immunity. We have shown that activation of NK-cells with cytokines IL-12/IL-15/L-18, used to increase their cytotoxicity for adoptive therapy, induces a parallel upregulation of PD-L1, restraining local antigen-specific T-cells (*Diniz, STM 2022*). We therefore engineered NK-cells to express the chemokine receptor CXCR6 and promote their homing to HCC and to secrete anti-PD-L1 antibodies to counteract their checkpoint inhibition of T-cells. We confirmed induction of CXCR6 expression on primary human NK-cells and their migration towards the prototypic HCC C-X-C-motif chemokine ligand-16 (CXCL16), indicating that the encoded chemokine receptor was functional. CXCR6 upregulation favoured NK-cell migration towards, and subsequent killing of, hepatoma cells in 2D and 3D *in vitro* systems. Production of anti-PD-L1 was established by its capacity to block PD-L1 *in cis* on NK-cells and *in trans* on hepatoma cells, abrogating staining of this ligand. Their release of anti-PD-L1 antibodies converted cytokine-activated NK-cells into predominant 'helpers', able to boost CD8⁺T-cell responses to HBV peptide stimulation or HBsAg⁺HepG2 cells. *In vivo* data indicate superior tumour control by CXCR6/anti-PD-L1-expressing NK-cells in a subcutaneous HCC mouse model, which is now being further investigated in an orthotopic setting. In summary, we genetically engineered cytokine-activated NK-cells to have enhanced homing within liver tumours, to mediate direct anti-tumour activity and act as a tumour-localised source of anti-PD-L1 antibodies to block this exhaustion pathway, thereby releasing their helper function to boost antigen-specific T-cells. This approach should promote the dual anti-tumour functionality of NK-cells and T-cells, to work in tandem against HCC.

Keywords: NK cell adoptive therapy; Cancer; PD-L1.

2854 - Category: TUMOR IMMUNOLOGY (TU)

Circulating monocytes from newly diagnosed multiple myeloma patients display a pro-inflammatory gene signature

DA SILVA, V.J.¹; OVIDER, I.C.¹; FOGOLIN, C.S.²; FILGUEIRAS, I.S.¹; ORNELLAS, F.¹; GUSMAO, A.F.¹; CABRAL-MARQUES, O.¹; MARTINEZ, G.A.³; SEGURO, F.S.³; ALMEIDA, R.R.¹; DE OLIVEIRA, T.G.M.¹; COUTO, S.C.F.¹; BARBUTO, J.A.M.²; ROCHA, V.G.³; RAMOS, R.N.³. 1. FMUSP, SÃO PAULO - SP - BRASIL; 2. ICB, SÃO PAULO - SP - BRASIL; 3. HCFMUSP, SÃO PAULO - SP - BRASIL.

Introduction: Multiple Myeloma (MM) is a hematological malignancy marked by the expansion of monoclonal plasma cells and systemic effects on the immune system. Prior studies from our group in solid tumors have shown that tumor-derived factors can modulate circulating myeloid cells. Here, we investigated the molecular and functional profile of circulating monocytes in newly diagnosed MM (NDMM) patients. **Methods:** PBMCs from healthy donors (HD, n = 20) and NDMM patients (n = 30) were collected. Monocytes were immunophenotyped by flow cytometry and differentiated into macrophage subsets (Mø-M1, Mø-M2) and moDCs *in vitro*. Expression of surface markers (HLADR, CD14, CD16, CD64, CD86, PDL1, CD163) was evaluated. FACs-Sorted HLADR⁺CD14⁺ monocytes were subjected to gene expression profiling using nCounter. **Results:** We observed a reduced frequency of CD14⁺⁺CD16⁻ and CD14⁺CD16⁺ subsets, with increased PDL1 in CD14^{dim}CD16^{high} monocytes from MM patients. *In vitro*, NDMM monocytes exhibited elevated CD64 (Mø-M1) and CD163 (Mø-M2), suggesting altered differentiation. Gene profiling revealed upregulation of pro-inflammatory genes (CXCL10, IL6, TNF, CCL4, CCL20, IFITM1) and downregulation of DPP4 and TNFSF4, indicating impaired immune regulation. Gene enrichment analysis highlighted pathways linked to chemotaxis, leukocyte migration, LPS response, and adhesion. Comparison with public datasets confirmed a shared inflammatory signature, particularly involving IL1B, TNF, IL6, and CXCL10, overlapping with MM tumor-conditioned monocytes (GSE143583). **Conclusion:** Circulating monocytes from NDMM patients exhibit inflammatory reprogramming and impaired regulatory features, impacting their differentiation potential. This inflammatory signature may serve as a potential biomarker for MM patients. **Funding:** CNPq, CAPES, Serrapilheira, PRONON.

Keywords: MONOCYTES; MULTIPLE MYELOMA; Bioinformatics.

2889 - Category: TUMOR IMMUNOLOGY (TU)

Infection-induced regulatory T cell dysfunction in the gut restricts colorectal tumorigenesis

GONÇALVES, L.M.¹; DE ASSIS, J.B.¹; DA CONCEIÇÃO, L.B.¹; COELHO, M.P.S.¹; OLIVEIRA, B.D.C.¹; DA SILVA, G.W.¹; RODRIGUES, G.M.B.¹; DE ARAUJO, M.V.¹; DE ABREU, A.M.¹; AYUPE, M.C.²; SALGADO, C.L.³; CARDOSO, A.¹; ALVES, C.B.¹; DA FONSECA, D.L.M.²; MUXEL, S.M.⁴; DA SILVA, P.⁵; GUIMARÃES, R.L.⁶; PEREIRA, B.T.N.⁶; VINOLO, M.A.R.⁶; DE OLIVEIRA, E.E.⁷; LEPIQUE, A.P.¹; DA FONSECA, D.M.¹. 1. INSTITUTE OF BIOMEDICAL SCIENCES - ICB/USP AND NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY FOR RESEARCH IN INFECTIOUS AND CHRONIC DISEASES OF THE MUCOSA AND SKIN - INCT MUCOSA AND SKIN, SÃO PAULO - SP - BRASIL; 2. INSTITUTE OF BIOMEDICAL SCIENCES - ICB/USP, SÃO PAULO - SP - BRASIL; 3. MAYO CLINIC, PHOENIX - ESTADOS UNIDOS DA AMERICA; 4. EACH-USP, SÃO PAULO - SP - BRASIL; 5. HARVARD UNIVERSITY, CAMBRIDGE - ESTADOS UNIDOS DA AMERICA; 6. UNIVERSITY OF CAMPINAS - UNICAMP, CAMPINAS - SP - BRASIL; 7. UNIVERSITY OF RIO DE JANEIRO STATE - UERJ, RIO DE JANEIRO - RJ - BRASIL.

Colorectal cancer (CRC) is one of the deadliest intestinal diseases, with nearly 1 million deaths and 2 million new cases annually, posing a major public health concern. Evidence suggests that infectious events disrupting intestinal immune homeostasis may influence CRC risk. Our research shows that resolving acute gastrointestinal infections, often seen as benign, leads to long-term remodeling of the immune and lymphatic systems in the gut and adjacent tissues like the mesentery. This results in chronic inflammation and impaired regulatory responses in the intestinal mucosa. We previously demonstrated that this altered environment increases susceptibility to inflammatory bowel diseases, prompting us to hypothesize a similar link with CRC. To investigate, we infected C57BL/6 mice with *Yersinia pseudotuberculosis* (YP) and, after bacterial clearance, induced CRC using azoxymethane and dextran sodium sulfate. Unexpectedly, YP-infected mice developed fewer tumors than uninfected controls, suggesting that prior infection may offer protection against cancer. Further analysis revealed that regulatory T cells (T_{regs}) in the colonic lamina propria of YP-infected mice were dysfunctional, unable to suppress inflammation *in vivo*, in colitis model, and *in vitro* assays. When we analyzed the fewer tumors that YP-infected mice developed, we found a significant reduction in tumor-infiltrating T_{regs}, accompanied by increased production of IFN- γ by TCD8⁺ and NK cells in the lamina propria. We propose that T_{reg} impairment enhances immune surveillance and limits tumor development, since functional T_{regs} are often co-opted by tumors to promote immune evasion. Post-infection, these T_{regs} fail to suppress, potentially shifting immune balance toward anti-tumor responses. These findings reveal a paradoxical role of infection-induced immune remodeling, suggesting that prior microbial exposure may reshape immunity and affect cancer risk, offering insights for prevention and immunotherapy.

Keywords: Colorectal Cancer; Prior gastrointestinal infection; Mucosal Immunology.

2895 - Category: TUMOR IMMUNOLOGY (TU)

Modified extracellular vesicles for STAT3 inhibition and activation of innate immune responses for cancer treatment

LESSO, J.A.L.¹; INOUE, M.G.S.²; DA CRUZ, L.L.P.²; RIBOVSKI, L.³; LEPIQUE, A.P.²; ZUHORN, I.S.³. 1. USP/UMCG, SÃO PAULO - SP - BRASIL; 2. USP, SÃO PAULO - SP - BRASIL; 3. UMCG, GRONINGEN - HOLANDA.

Cervical cancer remains a major health challenge in developing countries, primarily caused by persistent infection with high-risk human papillomaviruses. The viral oncogenes E6 and E7 activate NF κ B, leading to increased IL-6 expression and chronic STAT3 activation, which promote tumor progression and immune suppression. STAT3 signaling in myeloid cells contributes to immune tolerance. Our group previously demonstrated systemic STAT3 activation in cervical cancer patients, with tumor-derived extracellular vesicles (EVs) inducing phospho-STAT3 as a systemic effect of the disease. In this study, we evaluated the therapeutic potential of macrophage-derived EVs loaded with anti-STAT3 siRNA. Specifically, we investigated the internalization of EVs from differentiated and polarized THP-1 macrophages (M0, M1, M2, and undifferentiated) by tumor cells, as well as their differential effects on target cells. We found reduced phosphorylated STAT3 (p-STAT3) levels in M1-polarized macrophages compared to other subsets, identifying M1 as the most suitable EV donors for our approach. Among all conditions tested, EVs from M1 macrophages exhibited superior siRNA (Cy5-labeled) loading efficiency via sonication, with at least 30% incorporation. These EVs also showed higher expression of antigen presentation molecules, including HLA-DR, PD-L1, CD86, CD64, and CD80. Additionally, preliminary data indicated that treatment of HeLa cells with M1-derived siSTAT3-loaded EVs reduced STAT3 expression, decreasing STAT3-positive cells from approximately 30% to 5%.

Based on these findings, we propose M1 macrophage-derived EVs as a delivery platform for STAT3-targeted therapy in cervical cancer. This strategy may be extended to other malignancies driven by aberrant STAT3 activation and represents a novel immunomodulatory approach within EV-based therapies.

Keywords: EVs; Cervical cancer; STAT3.

2922 - Category: TUMOR IMMUNOLOGY (TU)

Characterization of the Involvement of the Transcriptional Cofactor IRF2BP2 in CD8 T Cell Proliferation

RAMOS, B.V.S.P.; VIEIRA, B.L.M.D.O.; VIOLA, J.. LABORATORY OF MOLECULAR IMMUNOLOGY, PROGRAM OF IMMUNOLOGY AND TUMOR BIOLOGY, NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

The adaptive immune response is dependent on T lymphocytes and is essential for maintaining homeostasis, also playing an important role in tumor immune response. IRF2BP2 (Interferon Regulatory Factor 2 Binding Protein 2) is a transcriptional repressor of NFAT1 protein, which regulates T cell activation. Previous findings from our group indicated that IRF2BP2 overexpression alters CD4 T cell activation and reduces proliferation. However, its role in CD8 T cells remains poorly understood. Our lab generated transgenic mice with T cell-specific overexpression of IRF2BP2 (LCK-Cre Rosa26 Stop-flox IRF2BP2-IRES-EGFP), hereafter referred to as IRF2BP2 T-KI. CD8 T cells from IRF2BP2 T-KI and control mice were purified, activated with anti-CD3 and anti-CD28, and cultured under effector differentiation conditions for five days, with daily expansion of culture area and volume. Total cell counts and viability were assessed on days 0, 2, 3, 4, and 5. On days 0 and 5, cells were also analyzed by flow cytometry for CD62L, CD44, Ki-67, and Caspase-3 expression. CD8 T cells from IRF2BP2 T-KI mice exhibited a progressive reduction in total cell number compared to controls, associated with a higher percentage of dead cells. Flow cytometry analysis at day 5 revealed reduced proliferative capacity (lower Ki-67 expression) and increased apoptosis (higher Caspase-3 levels) in IRF2BP2 T-KI CD8 T cells. IRF2BP2 impairs CD8 T cell expansion by simultaneously reducing proliferation and enhancing cell death. Further mechanistic studies and in vivo tumor models are necessary to elucidate its functional relevance in antitumor immunity.

Keywords: T lymphocytes;CD8 T cells;IRF2BP2.

2929 - Category: TUMOR IMMUNOLOGY (TU)

Cancer vaccines synergize with adoptively transferred T lymphocytes against solid tumors in a CXCR3-dependent manner

ALMEIDA, V.P.; MALAK, S.A.; WICKI, A.; MCAULIFFE, J.; NOBLECOURT, L.; PANETTI, S.; STEFFKE, E.; FERNANDES, R.; EYNDE, B.V.D.; LEUNG, C.. UNIVERSITY OF OXFORD, OXFORD - REINO UNIDO.

Adoptive T-cell therapy (ACT), which consists of systemic infusion of cytotoxic tumour-specific T cells to control neoplasias, still has limited efficacy against solid tumours in humans. Cancer vaccines hold promise to increase the clinical efficacy of ACT, but the mechanisms underlying this combination are still poorly understood. Here, we have combined ACT with cancer vaccines targeting tumor-specific antigens, using a vaccine platform based on heterologous prime-boost with two distinct viral vectors, the chimpanzee adenovirus ChAdOx1 and the poxvirus Modified Vaccinia Ankara (MVA). Compared to monotherapy controls, vaccines encoding for specific tumour antigens showed striking tumour regressions, which were correlated with increased expansion and infiltration of tumour-reactive T cells in tumors and lymphoid organs. Notably, this effect was primarily mediated by the priming injection of ChAdOx1, which triggered the release of CXCL10 in an antigen-independent manner and thereby promoted, through CXCR3, the early trafficking of adoptively transferred T cells into vaccine-draining lymph nodes. This innate immunity effect of ChAdOx1 allowed significant expansion of transferred T cells upon subsequent administration of the antigen-specific MVA boost. Our results highlight the key role of the ChAdOx1 vector in enhancing the recruitment of T cells to the lymph nodes via innate mechanisms, enabling an increased efficacy of the combination of cancer vaccines with ACT against solid tumors.

Keywords: Immunotherapy;Tumor immunology;Cytokines.

2966 - Category: TUMOR IMMUNOLOGY (TU)

Disruption of Pulmonary Immune Surveillance Following Intestinal Infection

DE ASSIS, J.B.¹; GONÇALVES, L.M.¹; DA CONCEIÇÃO, L.B.¹; COELHO, M.P.S.¹; DA SILVA, G.W.¹; DE ARAUJO, M.V.¹; OLIVEIRA, B.D.C.¹; RODRIGUES, G.M.B.¹; CARDOSO, A.¹; DE ABREU, A.M.¹; ALVES, C.B.¹; CAMARGO, L.¹; DE OLIVEIRA, E.E.²; LEPIQUE, A.P.¹; DA FONSECA, D.M.¹. 1. ICB/USP, SAO PAULO - SP - BRASIL; 2. UNIVERSITY OF RIO DE JANEIRO STATE, RIO DE JANEIRO - RJ - BRASIL.

Cancer is among the most lethal malignancies worldwide, influenced by environmental and immunological factors, including infection and chronic inflammation. Our group has shown that gastrointestinal infection with *Yersinia pseudotuberculosis* (YP) induces immune dysregulation and lymphatic remodeling in the mesentery, thereby sustaining local and systemic chronic inflammation. Conversely, infection-induced failure of intestinal regulatory mechanisms improves immune surveillance and protects against colorectal cancer (CRC). Based on these findings and supported by growing evidence of the interconnectedness among barrier immune compartments, we investigated whether such infection-induced intestinal immune imbalance also affects systemic immune surveillance in the lungs. Although YP-infected mice were protected against CRC, they developed lung tumors long-term after AOM/DSS treatment, a phenomenon absent in naïve controls. At six months post-induction, lung tumor frequency was reduced, but leukocyte infiltration remained high. In the urethane lung tumorigenesis model, assessed at 10 months post-injection, YP-infected mice exhibited increased pulmonary cell density, a higher frequency of Th17 cells, and reduced Tregs in the lung parenchyma. Additionally, increased expression of CD31 and Lyve-1 indicated enhanced angiogenesis and lymphangiogenesis. Notably, after the intratracheal instillation of Lewis Lung Carcinoma (LLC) cells, only YP-infected mice developed histopathological features of neoplasia. These findings suggest that YP infection, even long-term after bacterial clearance, predisposes the lung to tumor development by reshaping local immune and stromal environments, highlighting long-term effects of gastrointestinal infections on mucosal tissues. Ongoing experiments aim to elucidate the mechanisms mediating gut–lung crosstalk, advancing our understanding of how localized infections may reprogram systemic immunity and modulate cancer risk beyond the primary site.

Keywords: Gut-lung axis; *Yersinia pseudotuberculosis*; Mucosal immunology.

3016 - Category: TUMOR IMMUNOLOGY (TU)

GITRL COEXPRESSION ENHANCES CAR-T CELL RESISTANCE TO EXHAUSTION AND SUSTAINS ANTITUMOR ACTIVITY IN A GLIOBLASTOMA MODEL

FURTADO, I.P.¹; BRANDÃO, M.¹; ESTEVES, K.S.¹; DE LIMA, S.C.G.¹; ROSSETTI, R.¹; MARANINCHI, R.S.¹; MACHADO, C.B.¹; DE CARVALHO, J.V.¹; DE QUADROS, A.U.¹; FANTACINI, D.M.C.¹; SHIKU, H.²; COVAS, D.T.¹; DE SOUZA, L.E.B.¹. 1. HEMOCENTRO DE RIBEIRÃO PRETO, FMRP-USP, RIBEIRÃO PRETO - SP - BRASIL; 2. MIE UNIVERSITY, TSU - JAPÃO.

CAR-T cell therapy for solid tumors is hindered by the complex tumor microenvironment, which limits cell infiltration and sustained antitumor activity. Regulatory T cells are central to this suppression, dampening CAR-T cell effector functions. Based on previous reports, we hypothesized that the glucocorticoid-induced TNFR-related protein ligand (GITRL), through engagement of its receptor GTR, could potentiate T cell activity in CAR-T cells and overcome Treg suppression. In this study, we evaluated the antitumor efficacy of human anti-GD2 CAR-T cells coexpressing GITRL (CAR.GD2/GITRL) in a glioblastoma model. We generated CAR.GD2/GITRL and control CAR.GD2 cells and assessed their function during coculture with T98G glioblastoma cells (GD2+) and HCT166 control cells (GD2-) at 1:1 and 2:1 effector-to-target cell (E:T) ratios. Both constructs showed strong GD2-specific cytotoxicity, achieving ~90% lysis of T98G cells. Notably, upon serial tumor cell rechallenge, CAR.GD2/GITRL cells exhibited higher killing capacity, indicating superior functional persistence. Interestingly, while CAR.GD2 cells secreted more IFN- γ at a 1:1 E:T ratio, this advantage was lost at 2:1, suggesting CAR.GD2/GITRL cells were less sensitive to tumor cell burden. Phenotypic analysis of CAR-T cells recovered from cocultures revealed reduced expression of exhaustion markers in CAR.GD2/GITRL cells: 13% coexpressed PD-1 and LAG-3, compared to 38% in CAR.GD2 cells. In vivo, both CAR-T cell types significantly regressed T98G tumors and prolonged survival in NSG mice. These findings demonstrate that GITRL coexpression enhances CAR-T resistance to exhaustion and improves functional durability. This strategy may offer a broadly applicable approach to bolster the efficacy of CAR-T cell therapies against solid tumors.

Keywords: CAR-T; Solid tumors; GITRL.

3029 - Category: TUMOR IMMUNOLOGY (TU)

CBP/p300 inhibition enhances stem-like phenotype while limiting regulatory polarization in CAR-T cells against hematologic and solid tumors

CARVALHO, M.L.R.¹; CABRAL-PICCIN, M.P.¹; KINKER, G.S.¹; CAMBUI, R.A.G.¹; PEREIRA, J.¹; RONDINELLI, A.¹; ARAUJO, E.V.D.O.¹; ABDO, L.D.M.²; CORREIA, E.M.²; ARAGAO, E.A.A.²; GUSMAO, A.F.¹; LEMOS, M.M.M.¹; BATISTA, M.V.¹; SCHMIDT FILHO, J.¹; DE LIMA, V.C.C.¹; BONAMINO, M.H.²; MEDINA, T.D.S.¹. 1. A.C.CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 2. INSTITUTO NACIONAL DO CANCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

Chimeric Antigen Receptor (CAR)-T cell therapy has revolutionized the treatment of hematologic malignancies, leading to durable remissions in patients with B-cell acute lymphoblastic leukemia and non-Hodgkin lymphoma. However, a significant proportion of patients do not achieve complete responses, and the clinical efficacy of CAR-T cells against solid tumors remains limited. Major challenges include T cell exhaustion, the expansion of regulatory CAR-T cells (CAR-Tregs), and the immunosuppressive tumor microenvironment. We hypothesized that epigenetic modulation could promote a stem-like phenotype while limiting regulatory polarization in CAR-T cells. We first conducted a screening with different epigenetic inhibitors and identified CBP/p300 inhibition as the only approach capable of enhancing the stem-like phenotype and reducing the frequency of regulatory cells in healthy T cells, leading us to test the selective inhibitor A-485 during CAR-T cell manufacturing. In both anti-CD19 and anti-HER2 CAR-T cells, A-485 treatment enhanced and sustained the stem-like profile, reduced terminal differentiation and CAR-Treg development, increased the abundance of IFN γ producing TBET⁺ effector CAR-T cells with enhanced antitumor abilities, and downregulated inhibitory receptor markers. These effects persisted over time and under immunosuppressive conditions, such as exposure to TGF- β . RNA-seq analysis confirmed a transcriptional shift toward persistence-associated programs. Notably, A-485-treated CAR-T cells showed transcriptional profiles similar to those observed in B-ALL patients who respond to CAR-T therapy. Importantly, in patient-derived CAR-T cells, where A-485 also enhanced proliferation and anti-tumor activity in long-term killing and rechallenge assays against B-ALL and NHL cell lines. Altogether, our findings support CBP/p300 inhibition as a strategy to enhance CAR-T cell fitness, with translational potential for both hematologic and solid tumors.

Keywords: CAR-T cell therapy;Epigenetics;Hematologic and solid tumors.

3031 - Category: TUMOR IMMUNOLOGY (TU)

A Bead-Based Assay for the Screening of Monoclonal Antibodies That Disrupt PD 1/PD-L1 Interaction

PSCHIEDT, V.M.¹; GASSEN, R.B.¹; NUNES, J.E.S.¹; DE FREITAS, D.D.N.¹; BARTH JUNIOR, V.C.²; FROZZA, F.T.B.¹; NUNES, C.P.¹; BONORINO, C.¹. 1. UFCSPA, PORTO ALEGRE - RS - BRASIL; 2. DEPARTMENT OF MICROBIOLOGY, SÃO PAULO - SP - BRASIL.

The development of monoclonal antibodies (mAbs) targeting immune checkpoints is a cornerstone of modern immunotherapy. However, rapid and scalable methods to assess the blocking capacity of these mAbs are needed. While flow cytometry is a powerful tool, few assays have been described for this specific application. In this work, we report the development and validation of a bead-based flow cytometry assay to quantify the blockade of the programmed cell death protein 1 (PD-1) and its ligand (PD-L1) interaction. Recombinant human PD-1 protein was conjugated to polystyrene beads, and its interaction with recombinant human PD-L1 protein labeled with a fluorochrome was measured. In the presence of a non-blocking antibody (IgG control), the fluorescent PD-L1 binds to the PD-1 conjugated to the beads, resulting in a high geometric mean fluorescence intensity (gMFI). Conversely, when a blocking anti-PD-L1 mAb is pre-incubated with the fluorescent PD-L1, it binds to PD-L1, avoiding its interaction with the PD-1 on the beads. To develop the assay, an α -PD-L1 mAb biosimilar to Atezolizumab was used. The assay was validated for intra- and inter-assay precision and functionally confirmed using a T-cell co-culture assay with Expi 293 cells overexpressing PD-L1. The bead-based assay demonstrated dose-dependent inhibition by the α -PD-L1 mAb, with a calculated mean IC₅₀ of 3.12 μ g/mL. The method proved to be reproducible, no significant interaction effect was found between the runs and α PD-L1 mAb concentrations ($p = 0.1991$) as well as between three independent runs ($p = 0.2178$). In the functional co-culture system, the antibody significantly restored CD69 expression on the T-cell surface ($p < 0.0001$) at a concentration approximating the IC₅₀ detected by the bead-based assay. We have established a precise and robust bead-based assay that is a valuable tool for the functional screening of PD-1/PD-L1 blocking antibodies, supporting the development of new immunotherapies.

Keywords: Flow Cytometry;PD-L1;Immune Checkpoints Inhibitors.

3035 - Category: TUMOR IMMUNOLOGY (TU)

Multi-omic metabolic signatures for tumor immunity classification and prognosis

NOGUEIRA, H.A.C.; DE SOUZA, E.R.; LOPES, V.D.S.; MEDINA-ACOSTA, E.. UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE, CAMPOS DOS GOYTACAZES - RJ - BRASIL.

Tumor immune responsiveness is shaped by metabolic reprogramming, a hallmark of immune evasion, which directly impacts patient prognosis. In chimeric antigen receptor (CAR)-based immunotherapies (CAR-T, CAR-NK, and CAR-macrophage) tumor metabolism strongly influences efficacy. Competition for metabolites within the tumor microenvironment (TME) impairs effector immune cells and promotes regulatory or exhausted phenotypes. To investigate this dynamic, we developed a multi-omic pipeline to identify metabolic signatures—comprising enzyme-coding genes and their non-coding regulators—as potential biomarkers associated with immune phenotypes and clinical outcomes across 33 cancer types. These signatures were correlated with tumor phenotypes known to modulate immunotherapy response, including stemness, microsatellite instability (MSI), and tumor mutational burden (TMB). We assessed their association with the infiltration of 29 immune cell subtypes within the TME, allowing its classification as immunologically hot, cold, or variable. We identified 251,687 metabolic signatures distributed across carbohydrate (23.87%), lipid (23.32%), amino acid (22.38%), vitamin/cofactor (13.49%), nucleotide (6.17%), and energy (4.81%) pathways. Of these, 72% were associated with a cold immune phenotype, 18% with a hot phenotype, and 8% with variable profiles. Notably, 68% of the signatures were linked to stemness, predominantly displaying a cold immune classification. The *INMT* gene expression signature, related to selenium and tryptophan metabolism, was classified as hot in 13 tumor types. In lung adenocarcinoma, higher *INMT* expression correlated with reduced stemness and favorable prognosis. Conversely, its non-coding regulators—hsa-miR-30c-2-3p and hsa-miR-1293—were associated with increased risk and a cold immune phenotype. These findings available on CancerMetabolismGPS Shiny highlight the potential of multi-omic metabolic signatures to stratify tumor immunity and guide immunotherapy.

Keywords: Immune phenotype;Metabolic reprogramming;Multi-omic metabolic signatures .

3040 - Category: TUMOR IMMUNOLOGY (TU)

Creation of a platform to generate dendritic cells expressing Chimeric Antigen Receptors from human induced pluripotent stem cell to treat tumors

RIBEIRO, P.D.A.C.¹; DE SALLES, É.M.²; CATHARINO, L.C.C.¹; RALPH, A.C.L.²; ROCHA, V.G.³; DE OLIVEIRA, T.G.M.⁴; RAMOS, R.N.³. 1. INSTITUTE OF BIOMEDICAL SCIENCE OF UNIVERSITY OF SÃO PAULO (ICB-USP), SÃO PAULO - SP - BRASIL; 2. LABORATORY OF MEDICAL INVESTIGATION IN PATHOGENESIS AND DIRECTED THERAPY IN ONCO-IMMUNO-HEMATOLOGY (LIM-31), DEPARTAMENT OF HEMATOLOGY AND CELL THERAPY, HOSPITAL DAS CLÍNICAS HCFMUSP, SÃO PAULO - SP - BRASIL; 3. 1 LABORATORY OF MEDICAL INVESTIGATION IN PATHOGENESIS AND DIRECTED THERAPY IN ONCO-IMMUNO-HEMATOLOGY (LIM-31), DEPARTAMENT OF HEMATOLOGY AND CELL THERAPY, HOSPITAL DAS CLÍNICAS HCFMUSP, SÃO PAULO - SP - BRASIL; 4. FUNDAÇÃO PRÓ-SANGUE-HEMOCENTRO DE SÃO PAULO, SÃO PAULO, BRAZIL., SÃO PAULO - SP - BRASIL.

Immunotherapy based on T cells expressing Chimeric Antigen Receptor (CAR-T) has been successful to treat hematological tumors, while the same was not reported for solid tumors. Therefore, other immune cells have been explored to receive CARs. Dendritic cells (DC) are a heterogeneous population specialized in the antigen presentation, with great migratory capacity, being good candidates to receive CAR. Due to their scarcity in peripheral blood and low proliferative potential, an "off-the-shelf" source such as induced pluripotent cells (iPSCs) is needed to enable their use in cell therapy. Thus, our aim here is to develop iPSC-DCs expressing CAR as an alternative for solid tumors. We used the non-viral piggyBac transposon system to insert the CAR plasmid into the iPSC. After two rounds of cell sorting isolation and clone selection, we achieved purified CAR positive iPSCs, with a stable CAR expression over 40 days. iPSC differentiation into DCs followed classical established step-by-step phases: A) embryoid bodies obtention B) hematopoietic Stem-like cells C) immune myeloid-like cells. Using a combination of GM-CSF plus IL-4 after 30 days of differentiation, we obtained differentiated cells with a typical myeloid immune cell morphology, including few macrophage-like cells, with big cytoplasmatic vacuoles, and numerous cells with dendrite projections. Also, by multicolor flow cytometry we obtained cells expressing HLA-DR+CD64LowXCR1High phenotype, similarly to blood cDC1. Preliminary data shows that IPSC-derived cDC1-like cells induce CD3+ T cells proliferation in vitro such as monocyte-derived DCs, reinforcing their similarity with blood DCs. For the next steps, iPSC expressing CAR will be differentiated into cDC1-like to investigate their antitumor functions.

Keywords: Dendritic Cell;chimeric antigen receptor;Induced pluripotent Stem Cell.

3060 - Category: TUMOR IMMUNOLOGY (TU)

Linking baseline immune mediators and tumor microenvironment features to disease recurrence in colon cancer

SCARINI, J.F.¹; FIGUEIREDO, A.B.²; APOSTÓLICO, J.²; CORTES, L.G.F.²; IOHAN, L.¹; MESSIAS, I.M.²; BARONI, J.S.C.²; MORAIS, K.L.P.²; ROMANO, T.¹; USÓN JUNIOR, P.L.S.²; MOURA, F.²; SEID, V.E.³; PELEGRINO, K.²; MARTINELLI, M.²; CAMPREGHER, P.V.²; DE ALMEIDA, T.F.²; AMANCIO, T.T.²; FILIPPI, R.Z.²; GOLLOB, K.J.¹. 1. FACULDADE ISRAELITA DE CIÊNCIAS DA SAÚDE ALBERT EINSTEIN, HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 2. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL; 3. HOSPITAL MUNICIPAL GILSON DE CÁSSIA MARQUES DE CARVALHO, HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRASIL.

Colon cancer (CC) remains a leading cause of cancer-related mortality. While immunotherapy shows promise, variable response rates highlight the need for predictive biomarkers and novel therapeutic targets. We analyzed a prospective cohort of 37 treatment-naïve CC patients, stratified into disease-free (DF, n=25) and recurrence (DR, n=12) groups. Plasma levels of 32 immune mediators were quantified at four treatment timepoints (pre/post-surgery, pre/post-chemotherapy) using the MILLIPLEX® assay. Most tumors were right-sided, and MMR deficiency was more frequent in DR patients. Recurrence typically occurred within 12–16 months post-treatment. Baseline cytokine clustering showed no outcome-based separation; however, IL-1 α and IL-10 were elevated in DR patients and predicted recurrence (AUC=0.84). Cytokine network analysis revealed robust, coordinated Th1/lymphoid interactions in DF patients, persisting after treatment. In contrast, DR patients exhibited fragmented, innate-skewed cytokine networks. Single-cell RNA-sequencing (scRNA-seq, BD Rhapsody, WTA+VDJ) of tumor-infiltrating immune cells (n=6; 3 DF/3 DR) reflected the clinical diversity of the larger cohort, with representation of both stages III and IV and consistent recurrence patterns. All tumors were right-sided. MMR deficiency and more intensive chemotherapy regimens were observed among DR patients. scRNA-seq revealed increased monocytes and decreased B and CD8⁺ T cells in DR tumors. Differential gene expression showed downregulation of *RPS26*, *CTLA4*, *GZMB*, and *CXCR4* in T and B cell subsets of DF patients. TCR repertoire analysis indicated reduced T cell clonal diversity in DF patients, potentially reflecting a focused, effective anti-tumor response, whereas the higher diversity in DR patients may reflect broader, less effective immune activation. These findings underscore the prognostic value of longitudinal immune profiling and support targeting inflammatory and regulatory axes in advanced CC. Keywords: Colon cancer;Soluble analytes;scRNA-seq.

3077 - Category: TUMOR IMMUNOLOGY (TU)

Tumor compartment-specific shifts in macrophage phenotypes underlie distinct immune interactions in colorectal cancer

FILGUEIRAS, I.S.¹; FREITAS, D.G.A.²; QUAGLIATO, E.N.²; DE FREITAS, K.H.²; CABRAL-MARQUES, O.³; GALON, J.⁴; RAMOS, R.N.². 1. INSTITUTO DE CIÊNCIAS BIOMÉDICAS DA UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. D'OR INSTITUTE FOR RESEARCH AND EDUCATION, IDOR, SÃO PAULO - SP - BRASIL; 3. DEPARTMENT OF MEDICINE, DIVISION OF MOLECULAR MEDICINE, LABORATORY OF MEDICAL INVESTIGATION 29, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. INSERM, SORBONNE UNIVERSITÉ, UNIVERSITÉ DE PARIS, EQUIPE LABELLISÉE LIGUE CONTRE LE CANCER, CENTRE DE RECHERCHE DES CORDELIERS, LABORATORY OF INTEGRATIVE CANCER IMMUNOLOGY, PARIS - FRANÇA.

Recently, a concerning rise in colorectal cancer (CRC) diagnoses among younger individuals has emerged as a public health issue. Combined with the disease's substantial lethality, this trend underscores the urgency for improved early detection strategies and a better understanding of disease mechanisms. Macrophages have demonstrated a dual role in the tumor microenvironment, often debated due to their heterogeneous origins, functions, and distribution. A clearer characterization of these immune cells is essential to elucidate their role in tumor progression or control. Our study identified FOLR2⁺ macrophages as tissue-resident (TRMs) and TREM2⁺CADM1⁺ macrophages as monocyte-derived tumor-associated macrophages (TAMs), but their density, subset diversity, and spatial distribution in CRC remain unexplored. Using multiparametric flow cytometry in CRC patients we found TAMs enriched in tumors, while TRMs were more frequent in non-tumor areas. Multispectral immunohistochemistry was employed to examine spatial patterns of macrophage subsets and interactions with CD3⁺ cells across four anatomical zones: non-tumor tissue (NTT), stroma (ST), tumor margin (TM), and tumor center. Seven distinct subsets were identified among the combination of CD14, FOLR2 and TREM2 markers. FOLR2⁺ and CD14⁺FOLR2⁺ macrophages were enriched in non-malignant zones. Conversely, the tumor core showed predominance of TREM2⁺ and CD14⁺ cells, indicating a spatial shift in macrophage phenotype. Notably, FOLR2⁺ macrophages were more often near CD3⁺ cells in NTT than ST, despite greater overall density in the latter. In TM, their proximity to CD3⁺ cells decreased, while TREM2⁺ macrophages showed similar colocalization to FOLR2⁺ in NTT. These results support the concept that macrophage-lymphocyte interactions in CRC are influenced by tissue context, with distinct subsets exhibiting regionally confined patterns of association to T cells. This may impact tumor progression and, therefore, patient survival.

Keywords: Macrophages;Spatial Resolution;Tumor microenvironment.

3094 - Category: TUMOR IMMUNOLOGY (TU)

Tertiary Lymphoid Structures Drive Germinal Center B Cell Maturation and Tumor-reactive IgG in Renal Cancer

CAMBUI, R.A.G.; CARVALHO, M.L.R.; CABRAL-PICCIN, M.P.; ARAUJO, E.V.D.O.; DOS SANTOS, J.P.; GUSMAO, A.F.; ELIAS, B.D.D.S.; PIERRE, M.L.M.; DELECHIAVE, D.; DE LIMA, V.C.C.; KINKER, G.S.; MEDINA, T.D.S.. AC CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

In solid tumors such as clear cell renal cell carcinoma (ccRCC), B cells are particularly enriched within tertiary lymphoid structures (TLS), where they may undergo class switch recombination (CSR), differentiation, and affinity maturation. Although these features suggest the generation of tumor-specific antibodies, direct evidence remains limited. In this study, we compared B cells from TLS⁺ and TLS⁻ tumors in terms of distribution, functional status, and tumor specificity. Flow cytometry showed that TLS⁺ tumors had higher frequencies of germinal center B cells (GCBC), expressing BCL6, AID, CD95, and IgG. TLS⁺ tumors also showed more T follicular helper (Tfh) cells, fewer T follicular regulatory (Tfr) cells, and lower exhaustion marker expression in CD8⁺ T cells compared to TLS⁻ tumors. Histological analysis confirmed dense T/B cell aggregates, high density of IgG⁺ antibody-secreting cells (ASC), and strong correlation between B cell and ASC density, suggesting ongoing germinal center (GC)-like reactions in TLS⁺ tumors. Single-cell RNA sequencing (scRNA-seq) confirmed enrichment of GC transcriptional programs related to dark zone, light zone, CSR, and proliferation in TLS⁺ tumors. Functionally, B cells from TLS⁺ tumors differentiated into IgG⁺ ASC after co-culture with both autologous T cells or exogenous stimulation with CD40L, IL-21, and anti-BCR. CD40L was detected only in T cells from TLS⁺ tumors, supporting their role in B cell activation. TLS⁻ B cells failed to differentiate, even under exogenous stimulation. ELISA showed high IgG1, IgG2, and IgG3 in TLS⁺ cultures, while TLS⁻ produced mostly IgM. IgG from TLS⁺ cultures showed robust, selective binding to autologous tumor cells, as confirmed by flow cytometry and immunofluorescence. In contrast, TLS⁻ IgG showed negligible binding. Together, these findings indicate that TLS⁺ tumors support full GC reactions resulting in the production of class-switched, tumor-reactive IgG.

Keywords: Tertiary Lymphoid Structures; Germinal Center B Cells; Tumor-reactive IgG.

3221 - Category: TUMOR IMMUNOLOGY (TU)

Human CAR-engineered macrophages from Cord Blood Hematopoietic Stem Cell to treat HER2⁺ solid tumors

OVIDER, I.C.¹; DE CASTRO, L.F.¹; FOGOLIN, C.S.¹; DA SILVA, V.J.¹; COUTO, S.C.F.²; DE OLIVEIRA, T.G.M.²; ALMEIDA, R.R.¹; ROCHA, V.G.³; RAMOS, R.N.¹. 1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. PRO SANGUE - HEMOCENTRO DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 3. UNIVERSIDADE DE SÃO PAULO, SAO PAULO - SP - BRASIL.

Advances in the field of onco-immunology have highlighted the crucial role of macrophages within the tumor microenvironment. These cells exhibit key properties such as the ability to infiltrate dense tumor stroma, phagocytose, present tumor antigens, and secrete pro-inflammatory cytokines, features that make them promising candidates for cancer immunotherapy. However, macrophage-based therapies are limited by their poor proliferative capacity and inherent resistance to genetic modification due to their efficient recognition of foreign genetic material. To overcome these barriers, this study aimed to generate functional macrophages from umbilical cord blood-derived CD34⁺ hematopoietic stem cells (HSC) expressing anti-HER2 Chimeric Antigen Receptor (CAR), referred to as hscCARMacs, targeting their use in cancer immunotherapy. HSCs were isolated, expanded in vitro, and transduced with a lentiviral vector encoding a second-generation anti-HER2 CAR (hscCAR-aHER2). CAR⁺ cells were then differentiated into macrophages over 10 days in the presence of GM-CSF, M-CSF, and IL-3. hscCARMacs were evaluated for their morphology, immunophenotype, phagocytic activity, cytokine production and infiltration in three-dimensional tumor models. Following differentiation, 58.50 ± 30.52% of cells expressed CAR, displaying a CD34^{neg}CD14⁺HLA-DR⁺CD163⁺CD16⁺CD86⁺ phenotype and a typical macrophage morphology, with 100-fold increase in cell yield. Functionally, hscCARMacs demonstrated significantly enhanced in vitro phagocytosis of HER2⁺ tumor cell lines compared to non-transduced cells. Moreover, lentiviral transduction did not impair TNF-α production in response to LPS stimulation. In 3D culture models, hscCARMacs successfully infiltrated SKOV3 (HER2⁺) tumor spheroids and phagocytosed tumor cells, presenting a higher frequency than non-transduced Macrophages. Our findings support the potential application of hscCARMac as a relevant strategy for cellular immunotherapy against cancer.

Keywords: Immunotherapy; CAR-macrophage; HER2.

3299 - Category: TUMOR IMMUNOLOGY (TU)

Positive regulatory loop of Galanin/Galanin Receptor 2 (GALR2) and CCL2/CCR2 axes in Head and Neck Squamous Cell Carcinoma (HNSCC)

ALEMISEREJ, F.; REIS, I.B.; DE CARVALHO, M.M.; PELEGRIN, Á.F.; PASCHOAL, D.; NASCIMENTO, C.R.; FERREIRA, T.D.S.; ROSSA JR, C.. SÃO PAULO STATE UNIVERSITY, SCHOOL OF DENTISTRY, ARARAQUARA - SP - BRAZIL, ARARAQUARA - SP - BRASIL.

CCL2/CCR2 may influence tumor progression by various mechanisms, including increased tumor cell proliferation and invasiveness; by creating a tumor microenvironment with enhanced angiogenesis and through recruitment of immunosuppressive cells. Our study reveals that GALR2 overexpression in head and neck squamous cell carcinoma (HNSCC) cells promotes autogenous secretion of the chemokine CCL2 (MCP-1) and upregulates its receptor, CCR2, factors implicated in macrophage recruitment and M2-like polarization. RT-qPCR data confirm increased CCL2 expression in GALR2-overexpressing UM-SCC1 cells, which is significantly reduced upon treatment with the GALR2 antagonist M871. ELISA results using a cytokine array demonstrated elevated CCL2 secretion in UM-SCC22B cells and a marked decrease in SCC22B lines with CRISPR-mediated GAL or GALR2 silencing. Western blot analysis revealed higher CCR2 protein levels in UM-SCC1 cells overexpressing GALR2 compared to empty-vector controls. Additionally, our western blot results show that phosphorylated AKT (p-AKT) expression is elevated in UM-SCC1 cells overexpressing GALR2 compared to UM-SCC1-PCDNA controls and is reduced upon M871 treatment, suggesting activation of the AKT pathway downstream of GALR2 signaling. These findings indicate an autogenous positive regulatory loop involving the Gal/GALR2 and CCL2/CCR2 axes in HNSCC cells. This may affect not only neoplastic cell behavior and phenotype but also the infiltration and phenotype of macrophages in the tumor microenvironment with possible pro-tumoral effects.

Keywords: GALR2 signaling;CCL2/CCR2 axis;Head and neck squamous cell carcinoma (HNSCC).

3309 - Category: TUMOR IMMUNOLOGY (TU)

Epigenetic blockade of CBP/p300 reinvigorates exhausted TIL and boosts immune checkpoint blockade in metastatic melanoma

ELIAS, B.D.D.S.¹; GUSMAO, A.F.¹; CABRAL-PICCIN, M.P.¹; CARVALHO, M.L.R.¹; DELECHIAVE, D.¹; PIERRE, M.L.M.¹; PEREIRA, J.¹; CAMBUI, R.¹; ARAUJO, E.V.D.O.¹; NETO, J.P.D.¹; SILVA, M.J.D.B.E.¹; PINTOR, F.A.¹; KINKER, G.S.²; DE LIMA, V.C.C.¹; MEDINA, T.D.S.². 1. A.C.CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 2. UNIVERSITY OF SOUTHERN DENMARK, ODENSE - DINAMARCA.

Adoptive therapy with tumor-infiltrating lymphocytes (TIL) has recently been implemented as a clinical strategy for patients with relapsed or refractory metastatic melanoma. Despite its clinical efficacy, a significant proportion of patients either relapse or fail to achieve a robust response following adoptive transfer. The limited effectiveness of TIL therapy is thought to be associated with epigenetic mechanisms involved in T cell suppression and dysfunction. Here, we applied multiparametric flow cytometry to evaluate the effects of in vitro, epigenetic probe-mediated inhibition of the histone acetyltransferase (HAT) activity of the CREB-binding protein (CBP)/p300 complex in T cells from metastatic melanoma patients and a murine model of metastatic melanoma. Our results demonstrated that this epigenetic intervention consistently shifted exhausted CD4⁺ and CD8⁺ T cells from both the peripheral blood mononuclear cells of metastatic melanoma patients and the tumors of mice toward a less differentiated phenotype, reducing the frequency of cells expressing inhibitory receptors such as PD-1, CD39, LAG-3, TIGIT, and TIM-3 following chronic stimulation. Notably, CBP/p300 inhibition in vitro led to sustained granzyme B and interferon-gamma (IFN-γ) production by murine TIL and improved responsiveness to anti-PD-1 in both circulating T cells from patients and in the murine model. Collectively, our findings suggest that targeting CBP/p300 may be a promising strategy to reinvigorate exhausted TIL, enhance the efficacy of adoptive cell therapy, and improve responses to immune checkpoint blockade.

Keywords: Tumor infiltrating lymphocytes;Melanoma;Epigenetic therapy.

3353 - Category: TUMOR IMMUNOLOGY (TU)

Naturally occurring neutralizing anti-checkpoint antibodies predict survival in advanced lung cancer patients

FROZZA, F.T.B.¹; GASSEN, R.B.¹; FREITAS, D.¹; PSCHIEDT, V.M.¹; BAPTISTA, A.¹; NUNES, C.P.¹; DA FONTOURA, J.C.¹; MARQUES, C.G.D.S.¹; DE MATTOS, G.F.P.¹; LINS, M.P.²; SCHERER, M.M.¹; CALDEIRA, L.E.N.¹; MUHLEN, P.H.F.V.¹; DIEHL, C.¹; DA ROSA, S.A.¹; SODER, S.³; BONORINO, C.¹. 1. UFCSPA, PORTO ALEGRE - RS - BRASIL; 2. FEDERAL UNIVERSITY OF MATO GROSSO, CUIABÁ - MT - BRASIL; 3. IRMANDADE DA SANTA CASA DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL.

Immune checkpoint blockade has transformed cancer therapy, underscoring the critical role of immune surveillance in tumor control. Here, we identify a subset of treatment-naïve, pre-surgical cancer patients who harbor naturally occurring circulating IgG and IgA antibodies targeting immune checkpoints. Using single-cell sorting and B cell receptor (BCR) sequencing, we demonstrate that these antibodies originate from peripheral memory B cells and exhibit somatically mutated complementarity-determining regions (CDRs). Shared clonotypes are detectable in both draining lymph nodes and tumor tissues, consistent with antigen-driven selection and immune cell trafficking. Recombinant monoclonal antibodies derived from these B cells display high to intermediate affinity for checkpoint targets. They can functionally neutralize ligand-receptor interactions, restoring T cell activity *in vitro*. The presence of circulating anti-checkpoint antibodies correlates with dense tumor infiltration by B cells, CD8⁺ and memory CD4⁺ T cells, elevated PD-L1 expression in intratumoral CD45⁺ cells, in parallel with systemic signatures of T cell activation. Notably, these endogenous antibodies are predictive of improved survival in patients with advanced-stage lung cancer. These findings reveal a naturally occurring humoral response against checkpoint molecules, with potential implications for prognosis and therapeutic intervention.

Keywords: Tumor Immunology;Immune Checkpoint Blockade;Naturally-occurring anti-checkpoint antibodies.

3385 - Category: TUMOR IMMUNOLOGY (TU)

Generation of CD19 CAR-T Cells Using a Minicircle-Sleeping Beauty Transposon Platform

SILVA, L.R.B.¹; DOURADO, L.P.S.²; DOS REIS, S.A.²; ABDO, L.D.M.²; CORREIA, E.M.²; ARAGAO, E.A.A.²; BONAMINO, M.H.². 1. INCA/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL.

Chimeric Antigen Receptor T cell (CAR-T) immunotherapy targeting CD19 has demonstrated high response rates in patients with B-cell malignancies. Current CAR-T cell manufacturing involves genetic modification, activation, and *in vitro* expansion of autologous T lymphocytes. However, this process typically relies on viral vectors, which entail complex procedures and high costs, limiting widespread adoption. Recently, our group implemented a non-viral vector system using minicircle (MC) plasmids based on the Sleeping Beauty (SB) transposon to generate anti-CD19 CAR-T cells. MCs are simplified, non-viral DNA vectors derived from parental plasmids using engineered bacteria that express an inducible recombinase. This process generates vectors containing only the CAR transgene under a specific promoter, devoid of prokaryotic backbone sequences, thereby enhancing safety and compactness for gene delivery applications. In this study, we performed a comparative analysis of CD19 CAR-T cell generation using the SB transposon system delivered via either a MC or a conventional full plasmid (FP). PBMCs from healthy donors were isolated by density gradient centrifugation and enriched for T cells using anti-CD3 beads. Cells were nucleofected (4D-Nucleofector, Lonza) with PT4-19BBz (MC or FP format) and expanded in G-Rex plates with IL-2. Our results demonstrated that MC-derived CAR-T cells exhibited high viability ($78.35 \pm 8.73\%$) and robust CAR expression ($35.6 \pm 5.95\%$) after 8 and 12 days of culture. Starting with 5×10^6 total T cells, we obtained $1.7 \pm 0.8 \times 10^6$ CAR-positive cells. Notably, MC CAR-T cells showed improved CAR expression and expansion compared to those generated using the FP platform. Finally, *in vivo* antitumoral assays showed antitumor efficacy of MC CAR-T cells, comparable to FP CAR-T cells. The MC platform enables the generation of potent, anti-tumor CAR-T cells and supports scalable, cost-effective manufacturing suitable for point-of-care applications.

Keywords: CAR-T;leukemia;immunotherapy.

2652 - Category: VACCINES (VC)

UNCOVERING IMMUNE PROFILES IN YELLOW FEVER PATIENTS WITH PRIOR 17DD VACCINATION: INSIGHTS FROM THE 2016-2018 BRAZILIAN OUTBREAK

MENDES, M.J.¹; GONÇALVES, A.P.¹; ALMEIDA, L.T.¹; FRADICO, J.R.B.¹; PASCOAL-XAVIER, M.A.¹; CALZAVARA-SILVA, C.E.¹; MONATH, T.²; LABEAUD, A.D.³; DRUMOND, B.P.⁴; CAMPI-AZEVEDO, A.C.¹; MARTINS-FILHO, O.A.¹; CARVALHO, A.T.¹; ALVES, P.A.¹. 1. FIOCRUZ MINAS, BELO HORIZONTE - MG - BRASIL; 2. QUIGLEY BIOPHARMA LLC, LEOMINSTER - ESTADOS UNIDOS DA AMERICA; 3. STANFORD, STANFORD - ESTADOS UNIDOS DA AMERICA; 4. UFMG, BELO HORIZONTE - MG - BRASIL.

The 2016–2018 sylvatic yellow fever (YF) outbreaks in southeastern Brazil, particularly in Minas Gerais (MG), provided a unique opportunity to study immune responses in naturally infected individuals with prior vaccination. This study evaluated systemic and humoral responses in outbreak patients with documented recent (≤ 3 months) or longstanding (≥ 10 years) 17DD vaccination, and compared them to unvaccinated patients, 17DD vaccine recipients, and seronegative controls. Plaque reduction neutralization tests (PRNT) were conducted using both the 17DD vaccine strain and a wild-type (WT) yellow fever virus (YFV) isolate from the 2017–2018 MG outbreak. A 48-plex Luminex assay measured circulating pro-inflammatory and regulatory cytokines, chemokines, and growth factors. Samples from 10 vaccinated, naturally infected individuals (7 recent, 3 longstanding) were collected 30–80 days post-diagnosis. Humoral responses were heterogeneous: most individuals developed neutralizing antibodies (nAbs) against both YFV strains, with generally stronger responses to the WT virus. One recently vaccinated patient failed to develop detectable nAbs even 50 days post-vaccination and 40 days after symptom onset, while another exhibited exceptionally high nAb titers just 10 days post-onset, persisting over time. Analysis of soluble mediators is ongoing. Despite limited sample size, these rare cases offer valuable insights into YFV immunity in previously vaccinated individuals. Preliminary results highlight the complexity and variability of immune responses following WT YFV infection in this group, emphasizing the need for further studies on how prior vaccination, host factors, and natural infection interact to shape immune outcomes. Keywords: Yellow Fever Vaccine; Neutralizing antibodies; Soluble mediators.

2719 - Category: VACCINES (VC)

The Impact of Orthopoxvirus Vaccination and Mpox Infection on Cross-Protective Immunity

CRANDELL, J.¹; MONTEIRO, V.V.S.¹; PISCHEL, L.¹; FANG, Z.¹; MAIA, L.C.R.¹; ZHONG, Y.¹; LAWRES, L.²; ASSIS, G.M.²; DA SILVA, G.M.¹; ZALESKI, A.¹; DE LIRA, G.S.²; HIGA, L.²; BREBAN, M.¹; VOGELS, C.¹; CARIA, J.³; PINTO, A.R.³; ALMEIDA, V.³; MALTEZ, F.⁴; CORDEIRO, R.⁵; PÓVOAS, D.³; GRUBAUGH, N.¹; AOUN-BARAKAT, L.¹; GRIFONI, A.⁶; SETTE, A.⁶; CASTINEIRAS, T.M.²; CHEN, S.¹; YILDIRIM, I.¹; VALE, A.M.²; OMER, S.⁷; LUCAS, C.¹. 1. YALE UNIVERSITY, NEW HAVEN - ESTADOS UNIDOS DA AMERICA; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 3. HOSPITAL DE CURRY CABRAL, LISBON - PORTUGAL; 4. UNIVERSIDADE DE LISBOA, LISBON - PORTUGAL; 5. NATIONAL INSTITUTE OF HEALTH, LISBON - PORTUGAL; 6. LA JOLLA INSTITUTE FOR IMMUNOLOGY, LA JOLLA - ESTADOS UNIDOS DA AMERICA; 7. UNIVERSITY OF TEXAS SOUTHWESTERN, DALLAS - ESTADOS UNIDOS DA AMERICA.

The 2022 mpox outbreak marked a global resurgence of human orthopoxvirus infections, primarily driven by the emerging Clade IIb monkeypoxvirus (MPXV). Historically confined to Central and West Africa, Clade IIb MPXV's unprecedented transmission routes, such as sexual contact and sustained human-to-human spread, contributed to global spread and primarily affected younger, unvaccinated populations and men who have sex with men. Currently, no specific treatments exist for MPXV; during the 2022 outbreak, vaccinia virus-based smallpox vaccines, Dryvax and Jynneos, were used based on assumed cross-protection, but their effectiveness against Clade IIb MPXV remained uncertain. Our study addresses this important question by investigating how vaccinia-based smallpox vaccines confer immunity against the emerging poxvirus infections like Clade IIb MPXV. We conducted a comprehensive, multi-cohort analysis involving participants from the U.S., Brazil, and Portugal—including individuals vaccinated decades ago with Dryvax, recently vaccinated with Jynneos, boosted with Jynneos following previous Dryvax vaccination, and those infected with Clade IIb MPXV. We assessed systemic and mucosal immune responses, focusing on antibody and T cell immunity, and cross-reactivity among orthopoxviruses such as vaccinia, cowpox, and MPXV. Our findings indicate that both Dryvax and Jynneos induce cross-reactive antibodies and T cells, but neutralization capacity decreases with greater antigenic difference; Fc-mediated mechanisms like complement recruitment may partially compensate. Notably, T cell responses to orthopoxviruses persist up to 80 years post-Dryvax vaccination, whereas humoral immunity to MPXV wanes more rapidly without additional boosting. These findings deepen our understanding of smallpox vaccine-induced cross-protection and have important implications for guiding vaccination strategies worldwide, especially as more virulent strains like Clade Ib MPXV continue to emerge. Keywords: Emerging Viruses; B and T Cell Immunity; Immune Memory.

2740 - Category: VACCINES (VC)

Distinct Adjuvant Strategies and Antigen Multimerization Potentiate Immunity Induced by a SARS-CoV-2 RBD Subunit Vaccine

NUNES, J.P.D.S.¹; SILVA, M.D.O.²; APOSTOLICO, J.D.S.¹; DAHER, I.P.²; MARQUES, R.F.²; YAMAMOTO, M.M.²; CARVALHO, A.A.V.B.²; AMARANTE, M.F.D.C.²; DURIGON, E.²; WRENGER, C.²; JANINI, L.M.R.¹; DE SOUZA, E.E.²; SANTOS, R.A.¹; MARICATO, J.T.¹; CUNHA-NETO, E.³; KALIL, J.³; BOSCARDIN, S.B.²; ROSA, D.S.¹. 1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO (USP), SÃO PAULO - SP - BRASIL; 3. INSTITUTO DO CORAÇÃO (INCOR), SÃO PAULO - SP - BRASIL.

The rapid development of safe COVID-19 vaccines were critical for pandemic control. However, vaccine inequity and the emergence of new variants of concern highlighted major gaps in the global strategy to control SARS-CoV-2 infection. Most approved vaccines utilize the Spike protein as the main antigen due to its pivotal role in virus entry, mediated by the receptor binding domain (RBD). In this context, RBD stands out as a promising antigen for a subunit vaccine candidate, as it is the main target of neutralizing antibodies and has proven safety. In this study, we compared a Wuhan RBD monomer and homodimer with an RBD heterotrimer formulation composed of the Delta, Beta and Gamma variants. We also screened different adjuvants to optimize both humoral and cellular immunity. Our results showed that immunization with the RBD dimer and trimer, in the presence of the adjuvant AddaS03, elicited a higher humoral response and a broader neutralization profile. Additionally, RBD-trimer immunization more efficiently inhibited viral replication in the lungs of mice challenged with the ancestral Wuhan strain. We further optimized our vaccine formulation by combining the adjuvants AddaS03 and Poly I:C, which demonstrated a synergistic effect, integrating the potent humoral response induced by AddaS03 with the cellular Th1 skewing capacity of Poly I:C. The AddaS03+ Poly I:C mixture induced antibodies with higher affinity and an increased frequency of RBD-specific IgG2c-producing bone marrow plasma cells, highlighting the potential of this adjuvant combination to generate long-lived memory plasma cells. Additionally, we identified sequences within the RBD that induced specific IFN γ T cell responses. Peptide 12 emerged as the immunodominant CD4 T cell epitope, whereas peptides 28 and 29 successfully activated CD8 T cells. These findings underscore that antigen multimerization and the strategic combination of adjuvants can significantly improve vaccine immunogenicity. Keywords: Vaccine;SARS-CoV-2;Adjuvants.

3184 - Category: VACCINES (VC)

IDENTIFICATION OF RARE GENETIC VARIANTS IN INDIVIDUALS WITH SEVERE NEUROLOGICAL ADVERSE EVENTS FOLLOWING THE ADMINISTRATION OF ChAdOx1 nCoV-19 VACCINE IN BRAZIL

LOPES, M.R.G.¹; PIAZI, M.D.¹; CUNHA, D.P.¹; AGONIGI, B.N.S.¹; FERNANDES, A.R.²; VARGAS, F.R.³; DE OLIVEIRA, P.M.N.⁴; DE ALMEIDA, C.D.⁴; MAIA, M.D.L.D.S.⁴; MENDES-DE-ALMEIDA, D.P.⁵; FACCION, R.S.¹; DE VASCONCELOS, Z.F.M.¹. 1. INSTITUTO NACIONAL DE SAÚDE DA MULHER, DA CRIANÇA E DO ADOLESCENTE FERNANDES FIGUEIRA (IFF/FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL FLUMINENSE (UFF), RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO OSWALDO CRUZ (IOC/FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 4. INSTITUTO DE TECNOLOGIA EM IMUNOBIOLOGICOS (BIO-MANGUINHOS/FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 5. INSTITUTO NACIONAL DE INFECTOLOGIA EVANDRO CHAGAS (INI/FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

Introduction: The COVID-19 pandemic accelerated vaccine production and commercialization at an unprecedented pace. Pharmacovigilance activities were implemented to detect rare events supposedly attributable to vaccination or immunization, ensuring safety and effectiveness. Among the severe adverse events (SAEs) reported after the use of ChAdOx1 nCoV-19 vaccine in Brazil and worldwide were Guillain-Barré syndrome (GBS), acute disseminated encephalomyelitis (ADEM), and transverse myelitis (TM). The rarity of these immune-mediated events highlights the need to investigate underlying genetic factors.

Objective: We aim to identify rare genetic variants with potential biological relevance to neurological SAEs associated with ChAdOx1 nCoV-19 vaccine.

Method: A case series study with an associative objective. Whole blood samples from 18 cases — reported to the pharmacovigilance of Bio-Manguinhos/Fiocruz and from the database of Brazilian National Immunization Program — and 22 family members were collected for whole genome sequencing on the NovaSeq 6000 platform (Illumina). Genomic data were analyzed with Franklin (Qiagen), and functional enrichment analysis of genes with high/medium-confidence candidate variants (CVs) was performed with Enrichr to identify biological pathways potentially linked to immunopathogenic mechanisms. **Results:** A total of 77 CVs were identified in 42 genes previously associated with the above-mentioned neuropathies, inborn errors of immunity, SAEs to live attenuated virus vaccines, and/or demyelination. Enrichment analysis of 18 genes highlighted biological pathways related to TCR signaling and T cell activation, Th17 differentiation, and gene transcription via NF- κ B, ERK/MAPK, and JAK/STAT.

Conclusion: The genetic findings suggest that variants in genes involved in inflammation and autoimmunity may contribute to the neurological SAEs studied. Next steps include confirmation of low-confidence variants and functional assessment of CVs pathogenicity. Keywords: ChAdOx1 nCoV-19 vaccine;Vaccine-related severe adverse events;Genetic susceptibility.

3509 - Category: VACCINES (VC)

GLP Production and Characterization of the L3 Ribosomal Protein for a Universal Malaria Vaccine (UniMaV)

DE LACERDA, L.B.¹; GOMES, C.B.S.¹; RODRIGUES, B.C.M.¹; CORDEIRO, L.P.B.¹; ASSIS, B.R.D.²; GOMES, I.²; GOMEZ, D.²; TEIXEIRA, S.M.R.²; GAZZINELLI, R.T.²; JUNQUEIRA, C.F.¹. 1. FIOCRUZ - MINAS, BELO HORIZONTE - MG - BRASIL; 2. CTVACINAS, BELO HORIZONTE - MG - BRASIL.

Malaria remains a significant global health burden. *Plasmodium vivax* is a predominant malaria species in Latin America, often leading to recurrent outbreaks that hinder eradication efforts. While two approved vaccines target *P. falciparum*, their efficacy against severe disease and child mortality is limited. Therefore, developing a vaccine effective against multiple *Plasmodium* species is crucial to reduce the disease's impact. Our group identified a highly conserved ribosomal protein (L3) from *P. vivax* as a promising vaccine candidate. Initial studies demonstrated that the *P. vivax* L3 protein is recognized by CD8+ T cells after infection with multiple *Plasmodium* species, including *P. falciparum*, *P. knowlesi*, *P. cynomolgi*, and *P. yoelii*, which highlight its potential as a universal malaria vaccine. This study aimed to scale up its recombinant production under Good Laboratory Practice (GLP) conditions, characterize its physicochemical properties, formulate a vaccine. A master cell bank was established to ensure consistent L3 protein production. Preclinical studies demonstrated that L3 immunization induced a cross-species protective response against *P. yoelii*, reducing blood parasitemia by up to 60%. The initial GLP batch yielded 48 mg/L of highly purified L3 protein, meeting pharmacopeia requirements. The L3 protein exhibited stability at both -20°C and 5°C, maintaining its physicochemical properties for up to 9 months. To design the vaccine formulation, we performed pre-clinical assays to different adjuvants and protein concentrations, and the final formulation was set with a human-approved adjuvant (CTVad1) combined with 10 or 50 ug of the protein. These formulations are stable in 30 °C and 4 °C up to 3 months. The UniMaV demonstrates cross-species protective efficacy, combined with exceptional stability, positions it favorably for further development. Successful preclinical outcomes will pave the way for initiating clinical trials in Brazil.

Keywords: malaria vaccine;cross-species protection;T cell - based vaccine.

INDEX

Author	Author (full name)	Abstract code
., P.H.F.M.	PEDRO HENRIQUE F. MARCAL .	3219
.CAMARA, N.O.	NIELS O .CAMARA	3126
.CAMARA, N.O.	NIELS O .CAMARA	3348
.CAMARA, N.O.	NIELS O .CAMARA	3506
² ., ¹ .M.M.F.	¹ , MARCOS MACIEL FÉLIX ² .	3354
22/00291-4, S.M.M.F.	SANDRA MARCIA MUXEL FAPESP 22/00291-4	3008
22/00291-4, S.M.M.F.	SANDRA MARCIA MUXEL FAPESP 22/00291-4	3124
22/00291-4, S.M.M.F.	SANDRA MARCIA MUXEL FAPESP 22/00291-4	3139
22/00291-4, S.M.M.F.	SANDRA MARCIA MUXEL FAPESP 22/00291-4	3223
22/00291-4, S.M.M.F.	SANDRA MARCIA MUXEL FAPESP 22/00291-4	3230
ABBIATI, Y.	YASMIN ABBIATI	3505
ABDO, L.D.M.	LUIZA DE MACEDO ABDO	3029
ABDO, L.D.M.	LUIZA DE MACEDO ABDO	3113
ABDO, L.D.M.	LUIZA DE MACEDO ABDO	3142
ABDO, L.D.M.	LUIZA DE MACEDO ABDO	3153
ABDO, L.D.M.	LUIZA DE MACEDO ABDO	3171
ABDO, L.D.M.	LUIZA DE MACEDO ABDO	3332
ABDO, L.D.M.	LUIZA DE MACEDO ABDO	3385
ABDO, L.D.M.	LUIZA DE MACEDO ABDO	3443
ABDULKADER, F.R.D.M.	FERNANDO RODRIGUES DE MORAES ABDULKADER	2831
ABEDINI, A.	AMIN ABEDINI	3506
ABRAHÃO, M.M.	MARIA MUNIZ ABRAHÃO	3000
ABRAHÃO, M.M.	MARIA MUNIZ ABRAHÃO	3064
ABRANTES, L.S.	LETÍCIA SEABRA ABRANTES	2912
ABRANTES, L.S.	LETÍCIA SEABRA ABRANTES	2948
ABREU, J.P.D.S.	JULIANA PINHEIRO DOS SANTOS ABREU	2814
ABREU, K.E.D.S.	KENYA ELIZANA DE SOUZA ABREU	3073
ABUCATER, B.L.D.S.M.	BRENO LEONARDO DA SILVA MANSUR ABUCATER	3522
ABUNA, R.P.F.	RODRIGO PAOLO FLORES ABUNA	3428
ACIOLI, G.M.D.R.	GIOVANNA MARIA DE REZENDE ACIOLI	3119
ADAMI, F.L.	FLÁVIA LOPES ADAMI	3149
ADAMS, J.H.	JOHN H. ADAMS	2666
ADJAFRE, B.L.	BEATRIZ LIMA ADJAFRE	3022
ADRIANI, P.P.	PATRICIA PEREIRA ADRIANI	3450
AFONSO, S.L.	SOFIA L. AFONSO	2666
AGONIGI, B.N.D.S.	BRUNA NUNES DA SILVA AGONIGI	3268
AGONIGI, B.N.D.S.	BRUNA NUNES DA SILVA AGONIGI	3371
AGONIGI, B.N.S.	BRUNA N. S. AGONIGI	3174
AGONIGI, B.N.S.	BRUNA N. S. AGONIGI	3184
AGONIGI, B.N.S.	BRUNA N. S. AGONIGI	3243
AGRAWAL, A.	ANSHU AGRAWAL	3161
AGRAWAL, S.	SUDHANSHU AGRAWAL	3161
AGUDELO, J.S.H.	JUAN SEBASTIAN HENAO AGUDELO	2903
AKBAR, A.	ARNE AKBAR	2862
AKBAR, A.	ARNE AKBAR	2868
AKBAR, A.	ARNE AKBAR	3059
AKBAR, A.	ARNE AKBAR	3123
AKBAR, A.	ARNE AKBAR	3403
AKBAR, A.	ARNE AKBAR	3483
AKBAR, A.	ARNE AKBAR	3489
ALBERICI, L.C.	LUCIANE CARLA ALBERICI	3269
ALBIERO, I.	ISADORA ALBIERO	2817
ALBIERO, L.R.	LUCINÉIA REUSE ALBIERO	2737
ALBIERO, L.R.	LUCINÉIA REUSE ALBIERO	3092
ALBIERO, L.R.	LUCINÉIA REUSE ALBIERO	3322
ALBIERO, L.R.	LUCINÉIA REUSE ALBIERO	3398
ALBIERO, L.R.	LUCINÉIA REUSE ALBIERO	3448

ALBRECHT, L.	LETUSA ALBRECHT	2743
ALBRECHT, L.	LETUSA ALBRECHT	2836
ALBUQUERQUE, L.H.P.	LAWRENCE HENRIQUE PAZ ALBUQUERQUE	3523
ALCÂNTARA, D.A.D.S.	DANDARA ALANA DOS SANTOS ALCÂNTARA	3282
ALEMISEREJ, F.	FOUROUGH ALEMISEREJ	3013
ALEMISEREJ, F.	FOUROUGH ALEMISEREJ	3299
ALEMISEREJ, F.	FOUROUGH ALEMISEREJ	3301
ALENCAR, A.L.C.	ANA LUIZA CARNEIRO ALENCAR	3050
ALENCAR, A.L.C.	ANA LUIZA CARNEIRO ALENCAR	3355
ALESSIO, G.D.	GLAUCIA DINIZ ALESSIO	2630
ALEXANDRE, S.C.D.S.	SARAH CRISTINA DA SILVA ALEXANDRE	2955
ALISSON-SILVA, F.	FREDERICO ALISSON-SILVA	2724
ALLEGRETTI, S.M.	SILMARA MARQUES ALLEGRETTI	3255
ALLONSO, D.	DIEGO ALLONSO	3090
ALLONSO, D.	DIEGO ALLONSO	3095
ALMEIDA, A.	ANABELLA ALMEIDA	3219
ALMEIDA, A.P.G.	ANA PAULA GUARNIERI ALMEIDA	3081
ALMEIDA, A.P.G.	ANA PAULA GUARNIERI ALMEIDA	3082
ALMEIDA, A.P.G.	ANA PAULA GUARNIERI ALMEIDA	3102
ALMEIDA, A.P.G.	ANA PAULA GUARNIERI ALMEIDA	3116
ALMEIDA, A.P.G.	ANA PAULA GUARNIERI ALMEIDA	3162
ALMEIDA, B.D.S.	BIANCA DA SILVA ALMEIDA	2928
ALMEIDA, B.D.S.	BIANCA DA SILVA ALMEIDA	2984
ALMEIDA, B.D.S.	BIANCA DA SILVA ALMEIDA	3073
ALMEIDA, B.D.S.	BIANCA DA SILVA ALMEIDA	3149
ALMEIDA, D.V.	DALZIZA V. ALMEIDA	3240
ALMEIDA, D.V.	DALZIZA V. ALMEIDA	3260
ALMEIDA, F.	FAUSTO ALMEIDA	2808
ALMEIDA, F.B.D.R.	FAUSTO BRUNO DOS REIS ALMEIDA	3438
ALMEIDA, F.M.	FABRICIO MOREIRA ALMEIDA	2986
ALMEIDA, G.G.	GREGÓRIO GUILHERME ALMEIDA	2634
ALMEIDA, G.G.	GREGÓRIO GUILHERME ALMEIDA	2636
ALMEIDA, G.G.	GREGÓRIO GUILHERME ALMEIDA	2639
ALMEIDA, G.G.	GREGÓRIO GUILHERME ALMEIDA	2650
ALMEIDA, J.M.	JACIELLE MIRANDA ALMEIDA	2994
ALMEIDA, J.M.	JACIELLE MIRANDA ALMEIDA	2996
ALMEIDA, J.R.	JORGE REIS ALMEIDA	3006
ALMEIDA, L.T.	LETÍCIA TRINDADE ALMEIDA	2642
ALMEIDA, L.T.	LETÍCIA TRINDADE ALMEIDA	2644
ALMEIDA, L.T.	LETÍCIA TRINDADE ALMEIDA	2652
ALMEIDA, M.	MARCELO ALMEIDA	2862
ALMEIDA, M.	MARIANA ALMEIDA	2656
ALMEIDA, M.	MARIANA ALMEIDA	2664
ALMEIDA, M.	MARIANA ALMEIDA	2673
ALMEIDA, M.	MARIANA ALMEIDA	2676
ALMEIDA, M.D.	MARCELA DUTRA ALMEIDA	3026
ALMEIDA, N.	NATHALIE ALMEIDA	3294
ALMEIDA, P.P.	PALLOMA PORTO ALMEIDA	2925
ALMEIDA, R.D.N.	RAQUEL DAS NEVES ALMEIDA	2958
ALMEIDA, R.D.N.	RAQUEL DAS NEVES ALMEIDA	3001
ALMEIDA, R.R.	RAFAEL RIBEIRO ALMEIDA	2854
ALMEIDA, R.R.	RAFAEL RIBEIRO ALMEIDA	2855
ALMEIDA, R.R.	RAFAEL RIBEIRO ALMEIDA	3221
ALMEIDA, R.R.	RAFAEL RIBEIRO ALMEIDA	3236
ALMEIDA, V.	VASCO ALMEIDA	2719
ALMEIDA, V.P.	VINNYCIUS PEREIRA ALMEIDA	2929
ALOIA, T.P.A.	THIAGO PINHEIRO ARRAIS ALOIA	3293
ALVARES-SARAIVA, A.M.	ANUSKA MARCELINO ALVARES-SARAIVA	2879
ALVARES-SARAIVA, A.M.	ANUSKA MARCELINO ALVARES-SARAIVA	2902

ALVAREZ, C.M.C.	CLAUDIA MAGALHÃES CALVET ALVAREZ	3311
ALVAREZ, J.M.	JOSÉ MARIA ALVAREZ	2989
ALVAREZ, J.M.	JOSÉ MARIA ALVAREZ	3008
ALVES FILHO, J.C.F.	JOSE CARLOS FARIAS ALVES FILHO	2852
ALVES FILHO, J.C.F.	JOSE CARLOS FARIAS ALVES FILHO	3022
ALVES FILHO, J.C.F.	JOSE CARLOS FARIAS ALVES FILHO	3076
ALVES FILHO, J.C.F.	JOSE CARLOS FARIAS ALVES FILHO	3333
ALVES FILHO, J.C.F.	JOSE CARLOS FARIAS ALVES FILHO	3415
ALVES, B.H.	BRENO HENRIQUE ALVES	3265
ALVES, B.S.D.L.	BRENDA SARA DE LIMA ALVES	2729
ALVES, B.S.D.L.	BRENDA SARA DE LIMA ALVES	3104
ALVES, B.S.D.L.	BRENDA SARA DE LIMA ALVES	3180
ALVES, B.S.D.L.	BRENDA SARA DE LIMA ALVES	3438
ALVES, C.B.	CLARA BUENO ALVES	2734
ALVES, C.B.	CLARA BUENO ALVES	2735
ALVES, C.B.	CLARA BUENO ALVES	2741
ALVES, C.B.	CLARA BUENO ALVES	2878
ALVES, C.B.	CLARA BUENO ALVES	2889
ALVES, C.B.	CLARA BUENO ALVES	2938
ALVES, C.B.	CLARA BUENO ALVES	2966
ALVES, C.B.	CLARA BUENO ALVES	2967
ALVES, C.B.	CLARA BUENO ALVES	2982
ALVES, C.B.	CLARA BUENO ALVES	3076
ALVES, C.B.D.A.	CAROLINA BIFANO DE ASSIS ALVES	2808
ALVES, C.B.D.A.	CAROLINA BIFANO DE ASSIS ALVES	3084
ALVES, C.C.	CLARICE CARVALHO ALVES	2667
ALVES, C.C.D.S.	CAIO CÉSAR DE SOUZA ALVES	3046
ALVES, C.C.D.S.	CAIO CÉSAR DE SOUZA ALVES	3051
ALVES, C.F.	CHRISTIAN FERREIRA ALVES	3376
ALVES, C.R.B.	CRISTOFEM RAMOS BRAGA ALVES	2640
ALVES, D.C.D.A.	DANIELE COSTA DE ALMEIDA ALVES	2813
ALVES, D.C.D.A.	DANIELE COSTA DE ALMEIDA ALVES	2822
ALVES, D.C.D.A.	DANIELE COSTA DE ALMEIDA ALVES	3116
ALVES, E.	EDUARDO ALVES	3265
ALVES, É.A.R.	ÉRICA ALESSANDRA ROCHA ALVES	3032
ALVES, G.	GABRIEL ALVES	3135
ALVES, I.N.D.S.	ISABELLA NOGUEIRA DA SILVA ALVES	2825
ALVES, I.N.D.S.	ISABELLA NOGUEIRA DA SILVA ALVES	2830
ALVES, I.N.D.S.	ISABELLA NOGUEIRA DA SILVA ALVES	2859
ALVES, I.N.D.S.	ISABELLA NOGUEIRA DA SILVA ALVES	2864
ALVES, I.N.D.S.	ISABELLA NOGUEIRA DA SILVA ALVES	2888
ALVES, I.N.D.S.	ISABELLA NOGUEIRA DA SILVA ALVES	2901
ALVES, J.G.M.	JÚLIA GUIMARÃES MENDES ALVES	2699
ALVES, J.L.	JULIANA LIMA ALVES	2679
ALVES, J.L.B.	JOÃO LUCAS BENICIO ALVES	2759
ALVES, J.M.	JOÃO MANOEL ALVES	3269
ALVES, L.S.	LETICIA SILVA ALVES	2963
ALVES, L.S.	LETICIA SILVA ALVES	3253
ALVES, L.S.D.S.	LILIAN SANTOS DA SILVA ALVES	2797
ALVES, L.S.D.S.	LILIAN SANTOS DA SILVA ALVES	3006
ALVES, M.C.S.	MIRIAM COSTA SANTOS ALVES	3426
ALVES, M.R.	MARCELO RIBEIRO ALVES	2872
ALVES, M.R.	MARCELO RIBEIRO ALVES	3240
ALVES, M.R.	MARCELO RIBEIRO ALVES	3260
ALVES, M.S.	MILENA SANTOS ALVES	2909
ALVES, M.T.R.	MARCO TÚLLIO RODRIGUES ALVES	3032
ALVES, N.M.	NATÁLIA MUNHOZ ALVES	2958
ALVES, N.P.	NATÃ PORTUGAL ALVES	2737
ALVES, N.P.	NATÃ PORTUGAL ALVES	3092

ALVES, P.A.	PEDRO AUGUSTO ALVES	2642
ALVES, P.A.	PEDRO AUGUSTO ALVES	2644
ALVES, P.A.	PEDRO AUGUSTO ALVES	2652
ALVES, P.A.	PEDRO AUGUSTO ALVES	3294
ALVES, P.D.S.	PAULA DANIELA SOUZA ALVES	3135
ALVES, P.D.S.	PAULA DANIELA SOUZA ALVES	3462
ALVES, R.F.	RAFAELLA FERREIRA ALVES	2837
ALVES, R.F.	RAFAELLA FERREIRA ALVES	3055
ALVES, R.F.	RAFAELLA FERREIRA ALVES	3308
ALVES, R.F.	RAFAELLA FERREIRA ALVES	3310
ALVES, R.F.	RAFAELLA FERREIRA ALVES	3361
ALVES, S.Y.F.	SANDRA YASUYO FUKADA ALVES	3386
ALVES, T.C.D.L.	THAYNARA CASTELO DE LIMA ALVES	3445
ALVES, T.P.	TIAGO PIZZA ALVES	2794
ALVES, V.S.	VINÍCIUS SANTOS ALVES	3491
ALVES, W.A.	WENDEL ANDRADE ALVES	3140
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	2750
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	2755
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	2801
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	2970
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	3054
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	3088
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	3097
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	3170
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	3269
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	3337
ALVES-FILHO, J.C.	JOSÉ CARLOS ALVES-FILHO	3372
ALVES-SILVA, A.R.P.	ANA RUTH PAOLINETTI ALVES-SILVA	3143
AMAMURA, T.A.	THAIS AKEMI AMAMURA	2858
AMAMURA, T.A.	THAIS AKEMI AMAMURA	2990
AMAMURA, T.A.	THAIS AKEMI AMAMURA	3020
AMANCIO, T.T.	THIAGO TROLEZ AMANCIO	3060
AMANO, M.T.	MARIANE TAMI AMANO	3126
AMANO, M.T.	MARIANE TAMI AMANO	3388
AMARAL, B.S.	BRUNO SILVA AMARAL	2685
AMARAL, B.S.	BRUNO SILVA AMARAL	3316
AMARAL, B.S.	BRUNO SILVA AMARAL	3434
AMARAL, B.S.	BRUNO SILVA AMARAL	3451
AMARAL, E.H.B.	EDSON H B AMARAL	2833
AMARAL, E.H.B.	EDSON HENRIQUE BISPO AMARAL	2871
AMARAL, E.H.B.	EDSON HENRIQUE BISPO AMARAL	2887
AMARAL, E.H.B.	EDSON HENRIQUE BISPO AMARAL	2910
AMARAL, E.H.B.	EDSON HENRIQUE BISPO AMARAL	3025
AMARAL, E.H.B.	EDSON HENRIQUE BISPO AMARAL	3520
AMARAL, F.A.	FLAVIO ALMEIDA AMARAL	2681
AMARAL, F.A.	FLAVIO ALMEIDA AMARAL	2846
AMARAL, M.A.	MARIANA ABRANTES AMARAL	2784
AMARAL, M.P.	MARCELO PIRES AMARAL	3235
AMARAL, N.J.	NAYARA JULIA AMARAL	2665
AMARAL, N.J.	NAYARA JULIA AMARAL	2684
AMARANTE, M.F.	MARIA FERNANDA AMARANTE	3149
AMARANTE, M.F.D.C.	MARIA FERNANDA DE CASTRO AMARANTE	2740
AMARANTE-MENDES, G.P.	GUSTAVO P. AMARANTE-MENDES	2770
ANDERSEN, M.L.	MÔNICA LEVY ANDERSEN	3211
ANDRADE, B.D.B.	BRUNO DE BEZERRIL ANDRADE	3154
ANDRADE, C.M.	CAMILA MATTOS ANDRADE	3183
ANDRADE, C.M.	CAMILA MATTOS ANDRADE	3250
ANDRADE, C.M.	CAMILA MATTOS ANDRADE	3298
ANDRADE, C.M.	CAMILA MATTOS ANDRADE	3315

ANDRADE, C.M.	CAMILA MATTOS ANDRADE	3464
ANDRADE, E.B.D.S.	ELDER BARBOSA DA SILVA ANDRADE	3406
ANDRADE, J.	JULIA ANDRADE	3304
ANDRADE, J.C.S.	JESUANNE CARLA SILVA ANDRADE	2660
ANDRADE, J.D.S.	JENNYFER DE SOUZA ANDRADE	3458
ANDRADE, J.N.B.	JOÃO NILTON BARRETO ANDRADE	2809
ANDRADE, L.A.F.	LUIS ADAN FLORES ANDRADE	3281
ANDRADE, L.M.	LIDIA M ANDRADE	3140
ANDRADE, L.N.D.S.	LUCIANA NOGUEIRA DE SOUSA ANDRADE	3045
ANDRADE, M.F.	MARIANA FERRAZ ANDRADE	3051
ANDRADE, M.M.D.S.	MILENA MARY DE SOUZA ANDRADE	2767
ANDRADE-OLIVEIRA, V.	VINÍCIUS ANDRADE-OLIVEIRA	2784
ANDRADE-OLIVEIRA, V.	VINÍCIUS ANDRADE-OLIVEIRA	2909
ANDRADE-OLIVEIRA, V.	VINÍCIUS ANDRADE-OLIVEIRA	2917
ANDRADE-SILVA, M.	MAGAIVER ANDRADE-SILVA	3043
ANDRADE-SILVA, M.	MARCOS ANDRADE-SILVA	2874
ANDRADE-SILVA, M.	MARCOS ANDRADE-SILVA	2911
ANDREAZZI ¹ , A.E.	ANA ELIZA ANDREAZZI ¹ .	3354
ANDREEV, I.	ILYA ANDREEV	2783
ANDRIEUX, P.	PAULINE ANDRIEUX	3074
ANGELIM, M.K.	MONARA K ANGELIM	3365
ANGELIM, M.K.S.C.	MONARA KAÉLLE SÉRVULO CRUZ ANGELIM	2772
ANGELIM, M.K.S.C.	MONARA KAÉLLE SÉRVULO CRUZ ANGELIM	2809
ANGELIM, M.K.S.C.	MONARA KAÉLLE SÉRVULO CRUZ ANGELIM	2829
ANGELIM, M.K.S.C.	MONARA KAÉLLE SÉRVULO CRUZ ANGELIM	2831
ANGELIM, M.K.S.C.	MONARA KAÉLLE SÉRVULO CRUZ ANGELIM	2832
ANGELIM, M.K.S.C.	MONARA KAÉLLE SÉRVULO CRUZ ANGELIM	2863
ANIBAL, F.	FERNANDA ANIBAL	2819
ANIBAL, F.	FERNANDA ANIBAL	3255
ANIBAL, F.	FERNANDA ANIBAL	3297
ANSELMO, M.C.	MARIANA CARLONI ANSELMO	3255
ANSELMO-LIMA, W.T.	WILMA TEREZINHA ANSELMO-LIMA	3468
ANTONELLI, L.	LIS ANTONELLI	2636
ANTONELLI, L.	LIS ANTONELLI	2639
ANTONELLI, L.	LIS ANTONELLI	2713
ANTONELLI, L.	LIS ANTONELLI	2715
ANTONELLI, L.R.D.V.	LIS RIBEIRO DO VALLE ANTONELLI	2638
ANTONELLI, L.R.D.V.	LIS RIBEIRO DO VALLE ANTONELLI	2650
ANTONELLI, L.R.D.V.	LIS RIBEIRO DO VALLE ANTONELLI	2653
ANTONELLI, L.R.D.V.	LIS RIBEIRO DO VALLE ANTONELLI	2654
ANTONELLI, L.R.D.V.	LIS RIBEIRO DO VALLE ANTONELLI	2660
ANTONIO, M.B.	MARILIA BORDIGNON ANTONIO	3362
ANTONIO, N.D.S.	NAYARA DA SILVA ANTONIO	2950
ANTONIO, N.D.S.	NAYARA DA SILVA ANTONIO	2984
ANTONIO, N.D.S.	NAYARA DA SILVA ANTONIO	3073
ANTÔNIO, P.L.A.	PEDRO LUÍS ARAÚJO ANTÔNIO	3032
ANTUNES, B.M.	BARBARA MOURA ANTUNES	2866
ANTUNES, K.H.	KRIST HELEN ANTUNES	3321
ANTUNES, K.H.	KRIST HELEN ANTUNES	3435
ANTUNES, K.H.	KRIST HELEN ANTUNES	3455
ANTUNES, M.D.S.M.	MATEUS DA SILVA MATIAS ANTUNES	2870
ANTUNES, M.D.S.M.	MATEUS DA SILVA MATIAS ANTUNES	2964
ANTUNES, M.M.	MAÍSA MOTA ANTUNES	3305
ANTUNES, M.M.	MAÍSA MOTA ANTUNES	3334
ANTUNES, M.M.	MAÍSA MOTA ANTUNES	3433
AOKI, M.C.D.C.	MARIA CANDELARIA DE CASTRO AOKI	2840
AOKI, M.D.P.	MARIA DEL PILAR AOKI	2840
AOUN-BARAKAT, L.	LYDIA AOUN-BARAKAT	2719
APONTE, P.F.C.	PEDRO FERNADO CARO APONTE	3100

APOSTÓLICO, J.	JULIANA APOSTÓLICO	2976
APOSTÓLICO, J.	JULIANA APOSTÓLICO	3060
APOSTÓLICO, J.	JULIANA APOSTÓLICO	3314
APOSTÓLICO, J.	JULIANA APOSTÓLICO	3364
APOSTOLICO, J.D.S.	JULIANA DE SOUZA APOSTOLICO	2740
APOSTOLICO, J.D.S.	JULIANA DE SOUZA APOSTOLICO	2842
APOSTOLICO, J.D.S.	JULIANA DE SOUZA APOSTOLICO	3121
APRIGIO, F.	FELIPE APRIGIO	2837
APRIGIO, F.	FELIPE APRIGIO	3310
APRIGIO, F.	FELIPE APRIGIO	3361
ARAGÃO, D.M.D.O.	DANIELLE MARIA DE OLIVEIRA ARAGÃO	3213
ARAGÃO, D.R.	DYANE ROCHA ARAGÃO	3144
ARAGÃO, D.R.	DYANE ROCHA ARAGÃO	3242
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3029
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3108
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3113
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3142
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3153
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3332
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3385
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3443
ARAGAO, E.A.A.	EMMANUEL ARTHUR ALBUQUERQUE ARAGAO	3462
ARANTES, L.M.R.B.	LÍDIA MARIA REBOLHO BATISTA ARANTES	3392
ARAUJO, E.V.D.O.	EMMANUEL VINICIUS DE OLIVEIRA ARAUJO	2827
ARAUJO, E.V.D.O.	EMMANUEL VINICIUS DE OLIVEIRA ARAUJO	2841
ARAUJO, E.V.D.O.	EMMANUEL VINICIUS DE OLIVEIRA ARAUJO	3029
ARAUJO, E.V.D.O.	EMMANUEL VINICIUS DE OLIVEIRA ARAUJO	3094
ARAUJO, E.V.D.O.	EMMANUEL VINICIUS DE OLIVEIRA ARAUJO	3171
ARAUJO, E.V.D.O.	EMMANUEL VINICIUS DE OLIVEIRA ARAUJO	3309
ARAUJO, E.V.D.O.	EMMANUEL VINICIUS DE OLIVEIRA ARAUJO	3392
ARAÚJO, F.P.	FRANKLIN PEREIRA ARAÚJO	2692
ARAUJO, H.P.M.	HANNAH PAOLA MOTA ARAUJO	3494
ARAUJO, L.P.	LEANDRO PIRES ARAUJO	3348
ARAÚJO, R.K.S.	RENATO KENNEDY SOUZA ARAÚJO	2839
ARAUJO, R.N.	REINAN NASCIMENTO ARAUJO	2759
ARAÚJO, S.E.S.	SHEILA ESPÍRITO SANTO ARAÚJO	3110
ARAUJO, S.N.D.O.	SARA NUNES DE OLIVEIRA ARAUJO	2775
ARAUJO, S.N.D.O.	SARA NUNES DE OLIVEIRA ARAUJO	2833
ARAUJO, S.N.D.O.	SARA NUNES DE OLIVEIRA ARAUJO	3075
ARAUJO, S.N.D.O.	SARA NUNES DE OLIVEIRA ARAUJO	3125
ARAUJO, S.N.D.O.	SARA NUNES DE OLIVEIRA ARAUJO	3156
ARAUJO, S.N.D.O.	SARA NUNES DE OLIVEIRA ARAUJO	3318
ARAUJO, S.N.D.O.	SARA NUNES DE OLIVEIRA ARAUJO	3520
ARIAS-CARRASCO, R.	RAÚL ARIAS-CARRASCO	2724
ARIFA, R.D.D.N.	RAQUEL DUQUE DO NASCIMENTO ARIFA	2657
ARIFA, R.D.D.N.	RAQUEL DUQUE DO NASCIMENTO ARIFA	2675
ARIFA, R.D.D.N.	RAQUEL DUQUE DO NASCIMENTO ARIFA	3434
ARRUDA, A.C.	ALBERTO CARDOSO ARRUDA	3109
ARRUDA, M.S.P.	MARA SILVIA PINHEIRO ARRUDA	3109
ARRUDA, S.	SÉRGIO ARRUDA	2946
ARRUDA, S.M.	SÉRGIO MARCOS ARRUDA	3125
ASSIS, A.P.	ANA P. ASSIS	3269
ASSIS, B.R.D.	BRUNA RODRIGUES DIAS ASSIS	2641
ASSIS, B.R.D.	BRUNA RODRIGUES DIAS ASSIS	3509
ASSIS, G.M.	GUSTAVO MEIRA ASSIS	2719
ASSIS, G.M.	GUSTAVO MEIRA ASSIS	3085
ASSIS, M.	MARCELO ASSIS	3255
ASSIS, M.S.	MARIANNA SANTANA ASSIS	3282
ATAÍDE, N.	NAJARA ATAÍDE	2645

ATAIDES, L.S.	LAURA SANT'ANNA ATAIDES	3431
AUGUSTO, L.V.	LARA VILELA AUGUSTO	2962
AUGUSTO, L.V.	LARA VILELA AUGUSTO	2965
AUGUSTO, L.V.	LARA VILELA AUGUSTO	3003
AUGUSTO, P.S.D.A.	PAULO SERGIO DE ALMEIDA AUGUSTO	3408
AVELAR, T.M.T.	THALIA MEDEIROS TITO AVELAR	2797
AVELAR, T.M.T.	THALIA MEDEIROS TITO AVELAR	2810
AVELAR, T.M.T.	THALIA MEDEIROS TITO AVELAR	2816
AVELAR, T.M.T.	THALIA MEDEIROS TITO AVELAR	2995
AVELAR, T.M.T.	THALIA MEDEIROS TITO AVELAR	3006
AVELAR, T.M.T.	THALIA MEDEIROS TITO AVELAR	3136
AVELINO, S.E.P.	SOPHIA EDUARDA PEREIRA AVELINO	2729
AVELINO, S.E.P.	SOPHIA EDUARDA PEREIRA AVELINO	3104
AVELINO, S.E.P.	SOPHIA EDUARDA PEREIRA AVELINO	3180
AVELINO, S.E.P.	SOPHIA EDUARDA PEREIRA AVELINO	3438
AVILA, E.P.N.	ELDA PEREIRA NORONHA AVILA	3151
ÁVILA, T.V.	THIAGO VINICIUS ÁVILA	3102
ÁVILA, T.V.	THIAGO VINICIUS ÁVILA	3147
AYRIZONO, M.D.L.S.	MARIA DE LOURDES SETSUKO AYRIZONO	3067
AYUPE, M.C.	MARINA CAÇADOR AYUPE	2741
AYUPE, M.C.	MARINA CAÇADOR AYUPE	2878
AYUPE, M.C.	MARINA CAÇADOR AYUPE	2889
AYUPE, M.C.	MARINA CAÇADOR AYUPE	2944
AYUPE, M.C.	MARINA CAÇADOR AYUPE	2982
AZAMBUJA, D.	DANIEL AZAMBUJA	2880
AZEVEDO, A.C.C.	ANA CAROLINA CAMPI AZEVEDO	2638
AZEVEDO, I.R.	ISABELA RESENDE AZEVEDO	2928
AZEVEDO, I.R.	ISABELA RESENDE AZEVEDO	2950
AZEVEDO, I.R.	ISABELA RESENDE AZEVEDO	2984
AZEVEDO, I.R.	ISABELA RESENDE AZEVEDO	3073
AZEVEDO, I.R.	ISABELA RESENDE AZEVEDO	3149
AZEVEDO, L.G.	LORENA GARCIA AZEVEDO	3285
AZEVEDO, L.M.S.	LETÍCIA MARIA SOARES AZEVEDO	2685
AZEVEDO, L.M.S.	LETÍCIA MARIA SOARES AZEVEDO	3316
AZEVEDO, L.M.S.	LETÍCIA MARIA SOARES AZEVEDO	3434
AZEVEDO, L.M.S.	LETÍCIA MARIA SOARES AZEVEDO	3451
AZEVEDO, P.N.	PEDRO NOBRE AZEVEDO	2691
AZEVEDO, P.N.	PEDRO NOBRE AZEVEDO	2861
AZEVEDO, P.N.	PEDRO NOBRE AZEVEDO	3357
AZEVEDO, P.N.	PEDRO NOBRE AZEVEDO	3412
AZEVEDO, P.N.	PEDRO NOBRE AZEVEDO	3449
AZEVEDO, S.B.	SUANNE BURGOS AZEVEDO	3432
AZEVEDO, V.	VASCO AZEVEDO	2679
AZEVEDO, V.A.D.C.	VASCO ARISTON DE CARVALHO AZEVEDO	2684
AZEVEDO, V.A.D.C.	VASCO ARISTON DE CARVALHO AZEVEDO	2729
AZEVEDO-QUINTANILHA, I.G.	ISACLAUDIA GOMES AZEVEDO-QUINTANILHA	3480
AZEVEDO-QUINTANILHA, I.G.	ISACLAUDIA GOMES AZEVEDO-QUINTANILHA	3482
AZEVEDO-QUINTANILHA, I.G.	ISACLAUDIA GOMES AZEVEDO-QUINTANILHA	3490
BABÊTTO, A.M.	ADMA MACIEL BABÊTTO	3451
BACCAIN, I.B.	ISABELA BUQUIO BACCAIN	2783
BACHI, A.L.L.	ANDRÉ LUIS LACERDA BACHI	3419
BAFICA, A.L.B.	ANDRE LUIZ BARBOSA BAFICA	3288
BAGNO, F.F.	FLAVIA FONSECA BAGNO	2674
BAHIA, I.A.F.	IAN ANTUNES FERREIRA BAHIA	3008
BAHIA, I.A.F.	IAN ANTUNES FERREIRA BAHIA	3124
BAHIA, I.A.F.	IAN ANTUNES FERREIRA BAHIA	3139
BAHIA, I.A.F.	IAN ANTUNES FERREIRA BAHIA	3223
BALASSIANO, I.T.	ILANA TERUSZKIN BALASSIANO	3047
BALTHAR, Y.D.C.D.S.	YASMIN DE CASSIA DA SILVA BALTHAR	3036

BANIC, D.M.	DALMA MARIA BANIC	3355
BANZE, L.R.	LUCAS RAIMUNDO BANZE	2703
BANZE, L.R.	LUCAS RAIMUNDO BANZE	2704
BAO, S.N.	SÔNIA NAIR BAO	2763
BAO, S.N.	SÔNIA NAIR BAO	2764
BAO, S.N.	SÔNIA NAIR BAO	3001
BAPTISTA, A.	ANELISE BAPTISTA	3353
BAPTISTA, B.D.O.	BARBARA DE OLIVEIRA BAPTISTA	2743
BAPTISTA, B.D.O.	BARBARA DE OLIVEIRA BAPTISTA	2893
BAPTISTA, B.D.O.	BARBARA DE OLIVEIRA BAPTISTA	3050
BAPTISTA, B.D.O.	BARBARA DE OLIVEIRA BAPTISTA	3356
BAPTISTA, B.J.	BARBARA JOSÉ BAPTISTA	2849
BARAHUNA FILHO, P.F.R.	PAULO FRANCISCO REIS BARAHUNA FILHO	3474
BARBAS, C.	CORAL BARBAS	3139
BARBETA, M.G.D.O.	MARIA GABRIELA DE OLIVEIRA BARBETA	3121
BARBETA, M.G.D.O.	MARIA GABRIELA DE OLIVEIRA BARBETA	3134
BARBOSA, A.C.A.	ANA CLARA ANTONELLI BARBOSA	3103
BARBOSA, A.C.A.	ANA CLARA ANTONELLI BARBOSA	3190
BARBOSA, A.L.	ANA LUIZA BARBOSA	3465
BARBOSA, A.S.	ANGELA SILVA BARBOSA	2858
BARBOSA, A.S.	ANGELA SILVA BARBOSA	2990
BARBOSA, B.C.C.	BEATRIZ CRISTO CORRÊA BARBOSA	3107
BARBOSA, B.C.D.N.	BRUNA CARLOS DO NASCIMENTO BARBOSA	2898
BARBOSA, B.C.D.N.	BRUNA CARLOS DO NASCIMENTO BARBOSA	2905
BARBOSA, B.L.	BEATRIZ LIMA BARBOSA	3419
BARBOSA, C.E.A.	CELSO EDUARDO AMADOR BARBOSA	3474
BARBOSA, C.L.N.	CÉSAR LUIS NASCIMENTO BARBOSA	2698
BARBOSA, C.L.N.	CÉSAR LUIS NASCIMENTO BARBOSA	2699
BARBOSA, D.	DEIBS BARBOSA	3093
BARBOSA, E.R.M.D.C.	ERICA ROBERTA MACEDO DE CARVALHO BARBOSA	2704
BARBOSA, G.A.D.C.	GABRIEL ALBERTO DE CARVALHO BARBOSA	2750
BARBOSA, G.A.D.C.	GABRIEL ALBERTO DE CARVALHO BARBOSA	3088
BARBOSA, G.A.D.C.	GABRIEL ALBERTO DE CARVALHO BARBOSA	3097
BARBOSA, G.A.D.C.	GABRIEL ALBERTO DE CARVALHO BARBOSA	3170
BARBOSA, G.A.D.C.	GABRIEL ALBERTO DE CARVALHO BARBOSA	3415
BARBOSA, J.P.P.	JOÃO PAULO PEZZINI BARBOSA	2675
BARBOSA, J.P.P.	JOÃO PAULO PEZZINI BARBOSA	3434
BARBOSA, L.E.	LEILANE ESTRELA BARBOSA	3156
BARBOSA, L.M.P.	LUIZA MARQUES PASCHOAL BARBOSA	2635
BARBOSA, M.	MATHEUS BARBOSA	2772
BARBOSA, M.	MATHEUS BARBOSA	2829
BARBOSA, M.	MATHEUS BARBOSA	3034
BARBOSA, M.P.	MARCELA PAULINA BARBOSA	2981
BARBOSA, M.P.	MARCELA PAULINA BARBOSA	2993
BARBOSA, N.C.	NAYARA CARVALHO BARBOSA	3393
BARBOSA, N.C.	NAYARA CARVALHO BARBOSA	3494
BARBOSA, P.	PEDRO BARBOSA	2816
BARBOSA, S.C.	SARA CÂNDIDA BARBOSA	2766
BARBOSA, S.C.	SARA CÂNDIDA BARBOSA	3180
BARBOSA, V.	VITOR BARBOSA	2688
BARBOSA, Y.M.C.	YURI MÁRCIO CAMPOS BARBOSA	2676
BARBOSA-STANCIOLI, E.F.	EDEL FIGUEIREDO BARBOSA-STANCIOLI	2674
BARBOSA-STANCIOLI, E.F.	EDEL FIGUEIREDO BARBOSA-STANCIOLI	2692
BARBOZA, B.A.	BIANCA AMARAL BARBOZA	2927
BARBOZA, B.D.A.	BRUNA DE AMORIM BARBOZA	2862
BARBOZA, B.D.A.	BRUNA DE AMORIM BARBOZA	3489
BARBOZA, F.	FLÁVIO BARBOZA	3398
BARBOZA, F.	FLÁVIO BARBOZA	3448
BARBOZA, R.	RENATO BARBOZA	3290

BARBUTO, J.A.M.	JOSÉ ALEXANDRE MARZAGÃO BARBUTO	2762
BARBUTO, J.A.M.	JOSÉ ALEXANDRE MARZAGÃO BARBUTO	2854
BARBUTO, J.A.M.	JOSÉ ALEXANDRE MARZAGÃO BARBUTO	3024
BARBUTO, R.C.	RAFAEL CALVÃO BARBUTO	2645
BARCELLOS, J.F.M.	JOSE FERNANDO MARQUES BARCELLOS	2727
BARJA-FIDALGO, C.	CHRISTINA BARJA-FIDALGO	2925
BARONI, J.S.C.	JOSELANY SILVA CARVALHO BARONI	3060
BARONI, J.S.C.	JOSELANY SILVA CARVALHO BARONI	3314
BARONI, J.S.C.	JOSELANY SILVA CARVALHO BARONI	3364
BARRA, M.R.D.P.	MARIA RITA DE PAIVA BARRA	3267
BARRAL, A.M.P.	ALDINA MARIA PRADO BARRAL	3075
BARRAL, A.M.P.	ALDINA MARIA PRADO BARRAL	3125
BARRETO, T.M.M.	THÁIS M M BARRETO	2833
BARRIOS, A.	ANABELLA BARRIOS	2820
BARROS, B.A.F.	BARBARA AVELAR FERREIRA BARROS	2717
BARROS, C.D.C.O.	CLEYSON DA CRUZ OLIVEIRA BARROS	3472
BARROS, L.R.C.	LUCIANA RODRIGUES CARVALHO BARROS	3236
BARROS, M.	MARCIO BARROS	2972
BARROS, N.	NICOLE BARROS	2965
BARROS, P.H.A.	PEDRO HENRIQUE ARAGÃO BARROS	2875
BARROS, T.A.D.C.	TAMIRIS AZAMOR DA COSTA BARROS	3243
BARROS, T.A.D.C.	TAMIRIS AZAMOR DA COSTA BARROS	3249
BARROS, T.A.D.C.	TAMIRIS AZAMOR DA COSTA BARROS	3411
BARROSO, N.V.G.	NATÁLIA VELLOZO GARCIA BARROSO	2892
BARTH JUNIOR, V.C.	VALDIR CRISTOVAO BARTH JUNIOR	3023
BARTH JUNIOR, V.C.	VALDIR CRISTOVAO BARTH JUNIOR	3031
BARTOLOMEU, D.	DANIELLA BARTOLOMEU	2651
BASSAN, V.L.	VITOR LEONARDO BASSAN	3285
BASSI, V.	VITÓRIA BASSI	3079
BASSI, V.	VITÓRIA BASSI	3264
BASSI, V.	VITÓRIA BASSI	3375
BASSO, A.S.	ALEXANDRE SALGADO BASSO	3212
BASSO, A.S.	ALEXANDRE SALGADO BASSO	3348
BASSO, A.S.	ALEXANDRE SALGADO BASSO	3437
BASTOS, A.P.	ANA PAULA BASTOS	2780
BASTOS, A.P.	ANA PAULA BASTOS	2906
BASTOS, A.P.	ANA PAULA BASTOS	3096
BASTOS, A.P.	ANA PAULA BASTOS	3450
BASTOS, I.D.S.	IVANILDES DOS SANTOS BASTOS	3383
BASTOS, J.	JULIANA BASTOS	3331
BASTOS, M.R.	MANOELA RIBEIRO BASTOS	3135
BASTOS, R.P.D.S.	RANA PEREIRA DOS SANTOS BASTOS	3125
BASTOS, R.P.D.S.	RANA PEREIRA DOS SANTOS BASTOS	3156
BASTOS, R.P.D.S.	RANA PEREIRA DOS SANTOS BASTOS	3318
BASTOS, R.P.D.S.	RANA PEREIRA DOS SANTOS BASTOS	3520
BASTOS, V.C.	VICTORIA CÔRTEZ BASTOS	2963
BATAGLIOLI, B.G.C.	BIANCA GAZIERI CASTELUCCI BATAGLIOLI	2809
BATAGLIOLI, B.G.C.	BIANCA GAZIERI CASTELUCCI BATAGLIOLI	2920
BATAGLIOLI, B.G.C.	BIANCA GAZIERI CASTELUCCI BATAGLIOLI	3380
BATAH, S.	SABRINA BATAH	3130
BATAH, S.S.	SABRINA SETEMBRE BATAH	3465
BATISTA, M.H.Z.	MARIA HELENA ZECH BATISTA	2964
BATISTA, M.N.	MARIANA NOGUEIRA BATISTA	2857
BATISTA, M.V.	MARJORIE VIEIRA BATISTA	3029
BATISTA, M.V.	MARJORIE VIEIRA BATISTA	3171
BATISTA, R.B.	RAILDA BRANDÃO BATISTA	3406
BATISTA, V.L.	VIVIANE LIMA BATISTA	2686
BATISTA, V.L.	VIVIANE LIMA BATISTA	2988
BAUMLATT, A.P.	ANNA PAULA BAUMLATT	2742

BAVIER, A.C.D.	ANA CAROLINA DUARTE BAVIER	3306
BEASLEY, A.	ANACLARA BEASLEY	2957
BECERRA, A.D.M.	AMANDA DE MATOS BECERRA	3401
BECERRA, A.D.M.	AMANDA DE MATOS BECERRA	3465
BECK, B.K.	BIANCA KLAFKE BECK	3085
BECK, B.K.	BIANCA KLAFKE BECK	3107
BECKER, L.	LEV BECKER	2882
BEDOR, D.C.G.	DANILO CESAR GALINDO BEDOR	2972
BEHESHTI, A.	AFSHIN BEHESHTI	2691
BELARMINO, N.D.L.	NICOLLE DE LACERDA BELARMINO	2936
BELARMINO, N.D.L.	NICOLLE DE LACERDA BELARMINO	2948
BELITARDO, E.M.M.D.A.	EMÍLIA MARIA MEDEIROS DE ANDRADE BELITARDO	3197
BELLINAT, A.P.	ANA PAULA BELLINAT	2833
BELLIO, M.	MARIA BELLIO	2888
BELLIO, M.	MARIA BELLIO	3068
BELLO, G.	GONZALO BELLO	2872
BELLO, G.	GONZALO BELLO	3064
BELLO, H.J.S.	HORNBLENDA JOAQUINA SILVA BELLO	2782
BELLOZI, P.M.Q.	PAULA MARIA QUAGLIO BELLOZI	3001
BELÓRIO, E.P.	ELIZABETH PINTO BELÓRIO	3052
BELÓRIO, E.P.	ELIZABETH PINTO BELÓRIO	3057
BELTRÃO, G.S.	GABRIEL SALLES BELTRÃO	3387
BELTRÃO, G.S.	GABRIEL SALLES BELTRÃO	3391
BENEVIDES, L.	LUCIANA BENEVIDES	3158
BENEVIDES, L.	LUCIANA BENEVIDES	3372
BENEVIDES, L.	LUCIANA BENEVIDES	3375
BENEVIDES, L.	LUCIANA BENEVIDES	3376
BENEVIDES, L.	LUCIANA BENEVIDES	3428
BENGTON, M.	MARIO BENGTON	3004
BENICIO, I.	INGRA BENICIO	3520
BENJAMIM, C.F.	CLAUDIA FARIAS BENJAMIM	2728
BENJAMIM, C.F.	CLAUDIA FARIAS BENJAMIM	3422
BENJAMIM, C.F.	CLAUDIA FARIAS BENJAMIM	3473
BENTES, J.	JOSIANE BENTES	3070
BENTES, J.	JOSIANE BENTES	3109
BENTLEY, K.	KIRSTEN BENTLEY	2978
BENTO, C.A.D.M.	CLEONICE ALVES DE MELO BENTO	3062
BENTO, C.A.D.M.	CLEONICE ALVES DE MELO BENTO	3161
BENTO, L.P.F.	LAIS PEREIRA FERREIRA BENTO	3187
BENTO, N.P.C.	NATALY PERES CARVALHO BENTO	3093
BENTO, N.P.C.	NATALY PERES CARVALHO BENTO	3101
BERAZA, N.	NAIARA BERAZA	3220
BERBERT, L.	LUIZ BERBERT	2795
BERBERT, L.	LUIZ BERBERT	3150
BERÇOT, M.R.	MARCELO RODRIGUES BERÇOT	2809
BERÇOT, M.R.	MARCELO RODRIGUES BERÇOT	2882
BERÇOT, M.R.	MARCELO RODRIGUES BERÇOT	2920
BERÇOT, M.R.	MARCELO RODRIGUES BERÇOT	3252
BERÇOT, M.R.	MARCELO RODRIGUES BERÇOT	3380
BERÇOT, M.R.	MARCELO RODRIGUES BERÇOT	3487
BERGERO, G.	GASTÓN BERGERO	2840
BERGONZOLI, G.	GUSTAVO BERGONZOLI	2903
BERNARDES, W.P.D.O.S.	WILMA PATRICIA DE OLIVEIRA SANTOS BERNARDES	2646
BERNARDI, A.	ANDRESSA BERNARDI	2876
BERNARDI, J.D.M.	JÚLIA DE MOURA BERNARDI	3416
BERNARDINO, T.C.	THAISSA CONSONI BERNARDINO	3351
BERNARDINO, T.C.U.	TAINÁ CUNHA UDINE BERNARDINO	2778
BERTHO, A.L.	ALVARO LUIZ BERTHO	3050
BERTHO, A.L.	ALVARO LUIZ BERTHO	3356

BETBEDER, D.	DIDIER BETBEDER	2780
BEZERRA, J.D.A.	JAQUELINE DE ARAÚJO BEZERRA	3414
BEZERRA, M.R.L.	MARCUS RAFAEL LOBO BEZERRA	3103
BEZERRA, S.M.	STEPHANIA MARTINS BEZERRA	3171
BEZERRA, S.P.	SARAH PINHO BEZERRA	3044
BEZERRA, S.P.	SARAH PINHO BEZERRA	3069
BEZERRA, S.P.	SARAH PINHO BEZERRA	3313
BEZERRA, V.A.	VANESSA ALVARENGA BEZERRA	3314
BEZERRA, V.A.	VANESSA ALVARENGA BEZERRA	3364
BHATTARAI, B.	BISHAN BHATTARAI	2843
BIANCHI, J.	JAQUELINE BIANCHI	3255
BIANCHI, J.	JAQUELINE BIANCHI	3297
BIGGI, A.F.B.	ALISON FELIPE BORDINI BIGGI	2802
BIHNAM, D.	DANIEL BIHNAM	2935
BILANOVIC, J.	JANA BILANOVIC	3413
BILATE, A.M.	ANGELINA M. BILATE	3413
BILHÃO, R.	RAFAELA BILHÃO	3062
BISPO, R.	RAFAELA BISPO	3318
BITAR, R.	RENATA BITAR	2939
BITTENCOURT, M.E.G.E.D.A.G.	MARIA EDUARDA GABRICH ESTEVES DE ALCANTARA GOMES BITTENCOURT	2979
BIZARRO, H.D.D.S.	HELOÍSA D'AVILA DA SILVA BIZARRO	3352
BJØRNSDOTT, V.	VALGERDUR BJØRNSDOTT	3413
BLANCO, L.A.A.	LUIS ANDRES ARTEAGA BLANCO	2930
BLANK, M.	MARTINA BLANK	3387
BLANK, M.	MARTINA BLANK	3391
BOADA-ROMERO, E.	EMILIO BOADA-ROMERO	2808
BOAVENTURA, V.	VIVIANE BOAVENTURA	2862
BOAVENTURA, V.	VIVIANE BOAVENTURA	3125
BOAVENTURA, V.	VIVIANE BOAVENTURA	3156
BOAVENTURA, V.	VIVIANE BOAVENTURA	3318
BOAVENTURA, V.	VIVIANE BOAVENTURA	3508
BOAVENTURA, V.S.	VIVIANE SAMPAIO BOAVENTURA	3075
BOAVENTURA, V.S.	VIVIANE SAMPAIO BOAVENTURA	3307
BOBERMIN, D.M.	DAIANE MARA BOBERMIN	3288
BOCCA, A.L.	ANAMÉLIA LORENZETTI BOCCA	3079
BOCCA, A.L.	ANAMÉLIA LORENZETTI BOCCA	3264
BOCCA, A.L.	ANAMÉLIA LORENZETTI BOCCA	3375
BOEIRA, M.D.R.	MARIA CAROLINA DA ROSA BOEIRA	2826
BOFF, D.	DAIANE BOFF	2846
BOFF, D.	DAIANE BOFF	3244
BOLOTARI, M.	MARIANA BOLOTARI	3081
BOLOTARI, M.	MARIANA BOLOTARI	3162
BOM, A.P.D.A.	ANA PAULA DINIS ANO BOM	2743
BOM, A.P.D.A.	ANA PAULA DINIS ANO BOM	2778
BOM, A.P.D.A.	ANA PAULA DINIS ANO BOM	2812
BOM, A.P.D.A.	ANA PAULA DINIS ANO BOM	3154
BOM, A.P.D.A.	ANA PAULA DINIS ANO BOM	3159
BOM, A.P.D.A.	ANA PAULA DINIS ANO BOM	3243
BOM, A.P.D.A.	ANA PAULA DINIS ANO BOM	3249
BOM, A.P.D.A.	ANA PAULA DINIS ANO BOM	3411
BOMFIM, S.B.	SABRINA BORTOLOSSI BOMFIM	2777
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3029
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3048
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3108
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3113
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3135
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3142
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3144
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3153

BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3171
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3242
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3332
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3385
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3392
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3424
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3443
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3446
BONAMINO, M.H.	MARTIN HERNAN BONAMINO	3462
BONATO, V.L.D.	VÂNIA LUIZA DEPERON BONATO	3188
BONATO, V.L.D.	VÂNIA LUIZA DEPERON BONATO	3513
BONATTO, C.	CINTHIA BONATTO	3450
BONATTO, C.C.	CINTHIA C. BONATTO	2906
BONATTO, C.C.	CINTHIA C. BONATTO	3100
BONAVENTURA, D.	DANIELLA BONAVENTURA	2685
BONECINI, G.	GLORIA BONECINI	3058
BONECINI, G.	GLORIA BONECINI	3359
BONECINI, G.	GLORIA BONECINI	3404
BONECINI, G.	GLORIA BONECINI	3505
BONILLA, J.J.A.	JHON JHAMILTON ARTUNDUAGA BONILLA	3090
BONOMO, A.	ADRIANA BONOMO	3107
BONOMO, A.	ADRIANA BONOMO	3502
BONOMO, A.C.	ADRIANA CESAR BONOMO	2876
BONOMO, A.C.	ADRIANA CESAR BONOMO	2931
BONORINO, C.	CRISTINA BONORINO	2804
BONORINO, C.	CRISTINA BONORINO	3023
BONORINO, C.	CRISTINA BONORINO	3031
BONORINO, C.	CRISTINA BONORINO	3289
BONORINO, C.	CRISTINA BONORINO	3353
BONORINO, C.	CRISTINA BONORINO	3368
BONORINO, C.	CRISTINA BONORINO	3488
BORBA, P.	PEDRO BORBA	2779
BORBA, P.	PEDRO BORBA	3210
BORDIGNON, J.	JULIANO BORDIGNON	2857
BORDIGNON, J.	JULIANO BORDIGNON	3259
BORGES JUNIOR, E.V.	EDSON VICENTE BORGES JUNIOR	3282
BORGES, A.B.M.	AMANDA BUNN MONTEIRO BORGES	3399
BORGES, A.C.	ANA CLARA BORGES	3245
BORGES, A.C.N.	ANNA CRISTINA NEVES BORGES	3473
BORGES, A.O.	ANDREWS OLIVEIRA BORGES	3357
BORGES, A.O.	ANDREWS OLIVEIRA BORGES	3412
BORGES, L.A.B.	LIZE AMANDA BASAGLIA BORGES	2701
BORGES, M.B.J.	MARIA BEATRIZ JUNQUEIRA BORGES	3249
BORGES, M.B.J.	MARIA BEATRIZ JUNQUEIRA BORGES	3411
BORGES, M.M.	MONAMARIS MARQUES BORGES	3358
BORGES, M.P.	MARIA PEREIRA BORGES	2962
BORGES, S.G.	SOFIA GIACOMET BORGES	3321
BORGES, S.G.	SOFIA GIACOMET BORGES	3435
BORGES, S.G.	SOFIA GIACOMET BORGES	3455
BORGES, T.J.	THIAGO J. BORGES	2804
BORGES, T.M.	THALYA MÜLLER BORGES	2787
BORGES, V.	VALÉRIA BORGES	2862
BORGES, V.	VALÉRIA BORGES	3489
BORGES, V.M.	VINICIUS MARTINS BORGES	2781
BORONI, M.	MARIANA BORONI	2915
BORONI, M.	MARIANA BORONI	3111
BORTOLATTO, J.	JULIANA BORTOLATTO	3413
BORTOLINI, M.J.S.	MIGUEL JUNIOR SORDI BORTOLINI	2705
BORTOLUCI, K.R.	KARINA RAMALHO BORTOLUCI	2759

BORTOLUCI, K.R.	KARINA RAMALHO BORTOLUCI	3191
BORTOLUCI, K.R.	KARINA RAMALHO BORTOLUCI	3212
BORTOLUCI, K.R.	KARINA RAMALHO BORTOLUCI	3229
BORTOLUCI, K.R.	KARINA RAMALHO BORTOLUCI	3235
BOS, N.N.	NATALIA NOTARBERARDINO BOS	2975
BOS, N.N.	NATALIA NOTARBERARDINO BOS	3348
BOSCARDIN, S.B.	SILVIA BEATRIZ BOSCARDIN	2740
BOSCARDIN, S.B.	SILVIA BEATRIZ BOSCARDIN	2928
BOSCARDIN, S.B.	SILVIA BEATRIZ BOSCARDIN	2950
BOSCARDIN, S.B.	SILVIA BEATRIZ BOSCARDIN	2984
BOSCARDIN, S.B.	SILVIA BEATRIZ BOSCARDIN	3007
BOSCARDIN, S.B.	SILVIA BEATRIZ BOSCARDIN	3073
BOSCARDIN, S.B.	SILVIA BEATRIZ BOSCARDIN	3140
BOSCARDIN, S.B.	SILVIA BEATRIZ BOSCARDIN	3149
BOTELHO, M.A.	MONIQUE AMARAL BOTELHO	2874
BOTELHO, M.A.	MONIQUE AMARAL BOTELHO	2911
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	2856
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	2872
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	2898
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	2907
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	2930
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	2999
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	3009
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	3049
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	3393
BOU-HABIB, D.C.	DUMITH CHEQUER BOU-HABIB	3494
BOVI, W.G.	WESLEY GUIMARÃES BOVI	3454
BOVI, W.G.	WESLEY GUIMARÃES BOVI	3486
BOVI, W.G.	WESLEY GUIMARÃES BOVI	3493
BOVI, W.G.	WESLEY GUIMARÃES BOVI	3495
BOVI, W.G.	WESLEY GUIMARÃES BOVI	3497
BOVI, W.G.	WESLEY GUIMARÃES BOVI	3516
BOYLE, J.P.	JOSEPH P. BOYLE	3229
BOZZA, F.A.	FERNANDO AUGUSTO BOZZA	3036
BOZZA, F.A.	FERNANDO AUGUSTO BOZZA	3490
BOZZA, M.T.	MARCELO TORRES BOZZA	2818
BOZZA, M.T.	MARCELO TORRES BOZZA	2941
BOZZA, M.T.	MARCELO TORRES BOZZA	2963
BOZZA, M.T.	MARCELO TORRES BOZZA	2977
BOZZA, M.T.	MARCELO TORRES BOZZA	3086
BOZZA, M.T.	MARCELO TORRES BOZZA	3145
BOZZA, M.T.	MARCELO TORRES BOZZA	3166
BOZZA, M.T.	MARCELO TORRES BOZZA	3169
BOZZA, M.T.	MARCELO TORRES BOZZA	3253
BOZZA, M.T.	MARCELO TORRES BOZZA	3295
BOZZA, P.T.	PATRICIA TORRES BOZZA	3043
BOZZA, P.T.	PATRICIA TORRES BOZZA	3049
BOZZA, P.T.	PATRICIA TORRES BOZZA	3179
BOZZA, P.T.	PATRICIA TORRES BOZZA	3247
BOZZA, P.T.	PATRICIA TORRES BOZZA	3257
BOZZA, P.T.	PATRICIA TORRES BOZZA	3273
BOZZA, P.T.	PATRICIA TORRES BOZZA	3279
BOZZA, P.T.	PATRICIA TORRES BOZZA	3329
BOZZA, P.T.	PATRICIA TORRES BOZZA	3426
BOZZA, P.T.	PATRICIA TORRES BOZZA	3429
BOZZA, P.T.	PATRICIA TORRES BOZZA	3439
BOZZA, P.T.	PATRICIA TORRES BOZZA	3456
BOZZA, P.T.	PATRICIA TORRES BOZZA	3479
BOZZA, P.T.	PATRICIA TORRES BOZZA	3480

BOZZA, P.T.	PATRICIA TORRES BOZZA	3482
BOZZA, P.T.	PATRICIA TORRES BOZZA	3490
BOZZA, V.B.T.	VICENTE B. T. BOZZA	2941
BOZZA, V.B.T.	VICENTE B. T. BOZZA	2977
BRACCIALLI, M.	MARIANA BRACCIALLI	3510
BRAGA, F.H.P.	FLÁVIO HENRIQUE PARAGUASSÚ BRAGA	3142
BRAGA, L.D.C.	LETÍCIA DA CONCEIÇÃO BRAGA	2635
BRAGANHOL, E.	ELIZANDRA BRAGANHOL	2880
BRAGANHOL, E.	ELIZANDRA BRAGANHOL	3186
BRAGANHOL, E.	ELIZANDRA BRAGANHOL	3503
BRANCO, A.C.	ALEXANDRE C BRANCO	3219
BRANCO, J.M.C.R.	JESSICA MAGALHÃES COTIAS RISTOW BRANCO	2788
BRANCO, J.M.C.R.	JESSICA MAGALHÃES COTIAS RISTOW BRANCO	2960
BRANDÃO, B.P.	BÁRBARA PRENAZZI BRANDÃO	2897
BRANDÃO, B.P.	BÁRBARA PRENAZZI BRANDÃO	3195
BRANDÃO, H.D.M.	HUMBERTO DE MELLO BRANDÃO	2906
BRANDÃO, H.D.M.	HUMBERTO DE MELLO BRANDÃO	3450
BRANDÃO, M.	MILLENA BRANDÃO	2861
BRANDÃO, M.	MILLENA BRANDÃO	2870
BRANDÃO, M.	MILLENA BRANDÃO	2959
BRANDÃO, M.	MILLENA BRANDÃO	2969
BRANDÃO, M.	MILLENA BRANDÃO	3016
BRANDAO, S.G.	SARAH GUEDES BRANDAO	3454
BRANDAO, S.G.	SARAH GUEDES BRANDAO	3486
BRANDAO, S.G.	SARAH GUEDES BRANDAO	3493
BRANDAO, S.G.	SARAH GUEDES BRANDAO	3495
BRANDAO, S.G.	SARAH GUEDES BRANDAO	3497
BRANDAO, S.G.	SARAH GUEDES BRANDAO	3516
BRATTI, L.D.O.S.	LETÍCIA DE OLIVEIRA SOUZA BRATTI	3300
BRAUN, R.L.	RODRIGO LIGABUE BRAUN	3503
BRAZIL, C.R.F.	CARLOS RODOLPHO FERREIRA BRAZIL	2655
BREBAN, M.	MALLERY BREBAN	2719
BRENO, F.D.M.	FERNANDA DE MORAES BRENO	3085
BRÍGIDO, L.F.D.M.	LUÍS FERNANDO DE MACEDO BRÍGIDO	3002
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	2875
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	2949
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3053
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3103
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3127
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3135
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3144
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3152
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3190
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3242
BRIGIDO, M.D.M.	MARCELO DE MACEDO BRIGIDO	3389
BRILHANTE, L.M.V.	LÍVIA MARIA VIEIRA BRILHANTE	2983
BRIOSCHI, M.B.C.	MARIANA BORGES COSTA BRIOSCHI	3034
Brites, C.	CARLOS BRITES	3362
BRITO, D.C.	DOUGLAS C. BRITO	2891
BRITO, D.M.D.S.	DAVI MARCELO DOS SANTOS BRITO	2642
BRITO, D.V.	DIANA VIEIRA BRITO	3323
BRITO, J.	JOAQUIM BRITO	3219
BRITO, L.A.	LUCIANO A. BRITO	3093
BRITO, P.D.	PATRÍCIA DIAS BRITO	3404
BRITTO, A.C.D.C.	ANGELICA CRISTIANE DA CRUZ BRITTO	3314
BRITTO, A.C.D.C.	ANGÉLICA CRISTIANE DA CRUZ BRITTO	3364
BRITTO, G.D.S.G.	GABRIELA DE SALES GUERREIRO BRITTO	2833
BRITTO, G.D.S.G.	GABRIELA DE SALES GUERREIRO BRITTO	2871
BRITTO, G.D.S.G.	GABRIELA DE SALES GUERREIRO BRITTO	2910

BRITTO, G.D.S.G.	GABRIELA DE SALES GUERREIRO BRITTO	3025
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	2775
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3125
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3156
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3183
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3250
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3291
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3298
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3315
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3318
BRODSKYN, C.I.	CLAUDIA IDA BRODSKYN	3464
BROZINGA, P.F.A.	PAULA FERNANDES AGUIAR BROZINGA	2987
BRUNATO, M.L.	MAYRA LARISSA BRUNATO	3275
BRUNETTA, H.	HENVER BRUNETTA	2920
BRUNINI, G.L.	GUSTAVO LORIA BRUNINI	3088
BRYANT, C.	CLARE BRYANT	3229
BUCHRIESER, C.	CARMEN BUCHRIESER	3296
BUDNI, J.	JOSIANE BUDNI	3118
BUENO, F.F.	FABIO FERREIRA BUENO	2880
BUENO, F.V.D.S.	FILIPE VICENTE DOS SANTOS BUENO	3174
BUENO, F.V.D.S.	FILIPE VICENTE DOS SANTOS BUENO	3371
BULLA, A.C.S.	ANA CAROLINA SILVA BULLA	3095
BURGER, B.	BEATRIZ BURGER	2783
BURGER, B.	BEATRIZ BURGER	2869
BURGER, D.	DYLAN BURGER	3006
BUSO-LOPES, A.F.	ARIANE FIDELIS BUSO-LOPES	2733
BUTARELLI, A.L.D.A.	ANA LUIZA DE ARAÚJO BUTARELLI	2774
CABRAL, V.R.	VINICIUS RIBEIRO CABRAL	2758
CABRAL, Y.D.S.	YNGRID DOS SANTOS CABRAL	3151
CABRAL-MARQUES, O.	OTAVIO CABRAL-MARQUES	2854
CABRAL-MARQUES, O.	OTAVIO CABRAL-MARQUES	3077
CABRAL-PICCIN, M.P.	MARIELA PIRES CABRAL-PICCIN	2827
CABRAL-PICCIN, M.P.	MARIELA PIRES CABRAL-PICCIN	3029
CABRAL-PICCIN, M.P.	MARIELA PIRES CABRAL-PICCIN	3094
CABRAL-PICCIN, M.P.	MARIELA PIRES CABRAL-PICCIN	3171
CABRAL-PICCIN, M.P.	MARIELA PIRES CABRAL-PICCIN	3309
CABRAL-PICCIN, M.P.	MARIELA PIRES CABRAL-PICCIN	3428
CAÇADOR, M.A.	MARINA AYUPE CAÇADOR	2645
CAETANO, D.G.	DIOGO GAMA CAETANO	3064
CAETANO, G.P.	GABRIELA PRANDI CAETANO	2645
CAETANO, G.P.	GIOVANNA PATRÍCIO CAETANO	3051
CAL, T.C.M.F.	TEREZA CRISTINA MINTO FONTES CAL	3111
CALABRIA, P.A.L.	PAULA ANDREIA LUCAS CALABRIA	3218
CALABRIA, P.A.L.	PAULA ANDREIA LUCAS CALABRIA	3277
CALADO, R.	RODRIGO CALADO	3428
CALADO, R.T.	RODRIGO T. CALADO	2802
CALDAS, G.D.A.B.	GABRIELA DE ASSIS BURLE CALDAS	2651
CALDAS, G.D.A.B.	GABRIELA DE ASSIS BURLE CALDAS	2655
CALDAS, G.D.A.B.	GABRIELA DE ASSIS BURLE CALDAS	2672
CALDAS, G.D.A.B.	GABRIELA DE ASSIS BURLE CALDAS	3266
CALDEIRA, L.E.N.	LUIS EDUARDO NUNES CALDEIRA	3289
CALDEIRA, L.E.N.	LUIS EDUARDO NUNES CALDEIRA	3353
CALIARI, M.V.	MARCELO VIDIGAL CALIARI	2671
CALIXTO, G.F.G.	GABRIEL FELIPE GOMES CALIXTO	3044
CALJON, G.	GUY CALJON	3050
CALVACANTE, C.C.	CAROLINE CURIMBABA CALVACANTE	3294
CALVO, T.L.	THYAGO LEAL CALVO	3228
CALZAVARA-SILVA, C.E.	CARLOS EDUARDO CALZAVARA-SILVA	2644
CALZAVARA-SILVA, C.E.	CARLOS EDUARDO CALZAVARA-SILVA	2652

CALZAVARA-SILVA, C.E.	CARLOS EDUARDO CALZAVARA-SILVA	2653
CALZAVARA-SILVA, C.E.	CARLOS EDUARDO CALZAVARA-SILVA	2654
CÂMARA, A.C.	ANTÔNIA CLAUDIA CÂMARA	2972
CAMARA, N.O.S.	NIELS OLSEN SARAIVA CAMARA	2784
CAMARA, N.O.S.	NIELS OLSEN SARAIVA CAMARA	2909
CAMARA, N.O.S.	NIELS OLSEN SARAIVA CAMARA	2917
CAMARA, N.O.S.	NIELS OLSEN SARAIVA CAMARA	2975
CAMARA, N.O.S.	NIELS OLSEN SARAIVA CAMARA	3143
CÂMARA, P.H.M.	PEDRO HENRIQUE MEDEIROS CÂMARA	3296
CAMARGO, F.E.C.	FELIPPE EGON CASTRIGNANO CAMARGO	3089
CAMARGO, F.E.C.	FELIPPE EGON CASTRIGNANO CAMARGO	3091
CAMARGO, I.C.R.C.	ISADORA CELINE RODRIGUES CARNEIRO CAMARGO	3259
CAMARGO, L.	LARISSA CAMARGO	2734
CAMARGO, L.	LARISSA CAMARGO	2741
CAMARGO, L.	LARISSA CAMARGO	2878
CAMARGO, L.	LARISSA CAMARGO	2938
CAMARGO, L.	LARISSA CAMARGO	2966
CAMARGO, L.	LARISSA CAMARGO	2967
CAMARGOS, B.N.C.	BIANCA NATALIENE CARVALHO CAMARGOS	2717
CAMARGOS, I.R.	ISABELA RIBEIRO CAMARGOS	2756
CAMARGOS, I.R.	ISABELA RIBEIRO CAMARGOS	3267
CAMATTA, G.C.	GIOVANNA CALIMAN CAMATTA	2643
CAMATTA, G.C.	GIOVANNA CALIMAN CAMATTA	2645
CAMATTA, G.C.	GIOVANNA CALIMAN CAMATTA	2664
CAMATTA, G.C.	GIOVANNA CALIMAN CAMATTA	2676
CAMBUI, R.	RAYLANE CAMBUI	2827
CAMBUI, R.	RAYLANE CAMBUI	3171
CAMBUI, R.	RAYLANE CAMBUI	3309
CAMBUI, R.A.G.	RAYLANE ADRIELLE GONÇALVES CAMBUI	2841
CAMBUI, R.A.G.	RAYLANE ADRIELLE GONÇALVES CAMBUI	3029
CAMBUI, R.A.G.	RAYLANE ADRIELLE GONÇALVES CAMBUI	3094
CAMILLO, L.	LUCIANA CAMILLO	3297
CAMPI-AZEVEDO, A.C.	ANA CAROLINA CAMPI-AZEVEDO	2642
CAMPI-AZEVEDO, A.C.	ANA CAROLINA CAMPI-AZEVEDO	2644
CAMPI-AZEVEDO, A.C.	ANA CAROLINA CAMPI-AZEVEDO	2649
CAMPI-AZEVEDO, A.C.	ANA CAROLINA CAMPI-AZEVEDO	2652
CAMPI-AZEVEDO, A.C.	ANA CAROLINA CAMPI-AZEVEDO	2666
CAMPI-AZEVEDO, A.C.	ANA CAROLINA CAMPI-AZEVEDO	2667
CAMPI-AZEVEDO, A.C.	ANA CAROLINA CAMPI-AZEVEDO	3294
CAMPOLINA, B.G.	BRUNA GUERRA CAMPOLINA	2683
CAMPOS, A.J.L.	ANA JULIA LACERDA CAMPOS	3052
CAMPOS, A.T.P.	ANTONIO THIAGO PEREIRA CAMPOS	2831
CAMPOS, C.I.A.	CIBELE IRES APARECIDA CAMPOS	3165
CAMPOS, F.R.	FLORA ROSA CAMPOS	2956
CAMPOS, G.	GUILHERME CAMPOS	3294
CAMPOS, L.	LEA CAMPOS	2972
CAMPOS, M.C.S.	MARIA CLARA SALGADO CAMPOS	2795
CAMPOS, M.C.S.	MARIA CLARA SALGADO CAMPOS	2796
CAMPOS, M.C.S.	MARIA CLARA SALGADO CAMPOS	3145
CAMPOS, M.L.A.	MARIA LUISA ARANTES CAMPOS	2758
CAMPOS, M.M.	MARIANA MAGALHÃES CAMPOS	2906
CAMPOS, M.M.	MARIANA MAGALHÃES CAMPOS	3096
CAMPOS, M.M.	MARIANA MAGALHÃES CAMPOS	3100
CAMPOS, M.M.	MARIANA MAGALHÃES CAMPOS	3450
CAMPOS, R.M.P.	RAQUEL MARIA PEREIRA CAMPOS	3502
CAMPOS, T.L.	TÚLIO L CAMPOS	2833
CAMPREGHER, P.V.	PAULO V. CAMPREGHER	3060
CÂNDIDO, A.K.P.	ANA KAROLINA PEREIRA CÂNDIDO	2634
CANETTI, C.D.A.	CLAUDIO DE AZEVEDO CANETTI	2919

CANETTI, C.D.A.	CLAUDIO DE AZEVEDO CANETTI	3328
CANGUSSU, V.	VICTOR CANGUSSU	3248
CANGUSSU, V.	VICTOR CANGUSSU	3331
CANIATO, A.V.	AMANDA VERDICCHIO CANIATO	3191
CANIATO, A.V.	AMANDA VERDICCHIO CANIATO	3235
CANTARINI, D.G.	DÉBORAH GIOVANNA CANTARINI	2989
CANTARINI, D.G.	DÉBORAH GIOVANNA CANTARINI	3008
CANTO, F.	FÁBIO CANTO	2825
CANTO, F.	FÁBIO CANTO	2830
CANTO, F.	FÁBIO CANTO	2864
CANTO, F.	FÁBIO CANTO	2881
CANTO, F.	FÁBIO CANTO	2888
CANTO, F.	FÁBIO CANTO	3086
CANTONI, J.	JULIA CANTONI	2767
CANUTO, G.A.B.	GISELE ANDRÉ BAPTISTA CANUTO	2946
CAPELINI, C.E.	CARLOS EDUARDO CAPELINI	3468
CARA, D.C.	DENISE CARMONA CARA	2676
CARDOSO, A.	AMANDA CARDOSO	2734
CARDOSO, A.	AMANDA CARDOSO	2735
CARDOSO, A.	AMANDA CARDOSO	2741
CARDOSO, A.	AMANDA CARDOSO	2878
CARDOSO, A.	AMANDA CARDOSO	2889
CARDOSO, A.	AMANDA CARDOSO	2938
CARDOSO, A.	AMANDA CARDOSO	2966
CARDOSO, A.	AMANDA CARDOSO	2967
CARDOSO, A.	AMANDA CARDOSO	2982
CARDOSO, A.	AMANDA CARDOSO	3076
CARDOSO, C.A.A.	CLAUDETE APARECIDA ARAÚJO CARDOSO	2742
CARDOSO, C.R.D.B.	CRISTINA RIBEIRO DE BARROS CARDOSO	3303
CARDOSO, C.R.D.B.	CRISTINA RIBEIRO DE BARROS CARDOSO	3307
CARDOSO, C.R.D.B.	CRISTINA RIBEIRO DE BARROS CARDOSO	3381
CARDOSO, C.R.D.B.	CRISTINA RIBEIRO DE BARROS CARDOSO	3508
CARDOSO, E.C.	ELAINE CRISTINA CARDOSO	2733
CARDOSO, L.S.	LUCIANA SANTOS CARDOSO	3370
CARDOSO, L.S.	LUCIANA SANTOS CARDOSO	3432
CARDOSO, M.F.	MILENA FERNANDES CARDOSO	3232
CARDOSO, M.F.	MILENA FERNANDES CARDOSO	3399
CARDOSO, M.F.	MILENA FERNANDES CARDOSO	3418
CARDOSO, M.F.	MILENA FERNANDES CARDOSO	3484
CARDOSO, S.W.	SANDRA WAGNER CARDOSO	3000
CARDOSO, S.W.	SANDRA WAGNER CARDOSO	3064
CARDOSO, S.W.	SANDRA WAGNER CARDOSO	3105
CARDOSO, S.W.	SANDRA WAGNER CARDOSO	3240
CARDOSO, S.W.	SANDRA WAGNER CARDOSO	3260
CARDOSO, S.W.	SANDRA WAGNER CARDOSO	3362
CARDOSO, T.M.D.S.	THIAGO MARCONI DE SOUZA CARDOSO	2739
CARDOSO, T.M.D.S.	THIAGO MARCONI DE SOUZA CARDOSO	3370
CARDOZO, A.K.	ANDRÉIA KAYSER CARDOZO	2880
CARELS, N.	NICOLAS CARELS	2757
CARGNELUTTI, D.E.	DIEGO ESTEBAN CARGNELUTTI	3276
CARIA, J.	JOÃO CARIA	2719
CARLETTO, T.D.O.T.M.	TATIANE DE OLIVEIRA TEIXEIRA MUNIZ CARLETTO	3312
CARLI, A.D.P.	ALESSANDRA DE PAULA CARLI	3046
CARLI, A.D.P.	ALESSANDRA DE PAULA CARLI	3051
CARLOS, R.M.G.	RAISSA MAYARA GOMES CARLOS	3092
CARNEIRO, F.R.G.	FLAVIA RAQUEL GONCALVES CARNEIRO	2757
CARNEIRO, G.	GUILHERME CARNEIRO	3414
CARNEIRO, M.C.	MILENA CARVALHO CARNEIRO	2792
CARNEIRO, P.	POLLYANNA CARNEIRO	2954

CARNEIRO, V.L.	VALDIRENE LEÃO CARNEIRO	3312
CARNIO, E.C.	EVELIN CAPELLARI CARNIO	3165
CARRIÓN, F.	FEDERICO CARRIÓN	2957
CARROZZA, N.C.	NATHALLIA CAVALCANTI CARROZZA	3157
CARVALHO, A.A.V.B.	ALEXIA ADRIANNE VENCESLAU BRITO CARVALHO	2740
CARVALHO, A.F.S.	ANTÔNIO FELIPE S. CARVALHO	2661
CARVALHO, A.F.S.	ANTÔNIO FELIPE S. CARVALHO	2669
CARVALHO, A.M.	AUGUSTO M. CARVALHO	2921
CARVALHO, A.M.	AUGUSTO M. CARVALHO	3167
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2630
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2635
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2638
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2644
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2645
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2649
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2652
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2664
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2666
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2790
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	2972
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	3028
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	3294
CARVALHO, A.T.	ANDRÉA TEIXEIRA CARVALHO	3395
CARVALHO, B.D.O.	BIANCA DE OLIVEIRA CARVALHO	3287
CARVALHO, C.S.	CAROLINA SILVA CARVALHO	2833
CARVALHO, E.M.	EDGAR MARCELINO CARVALHO	2921
CARVALHO, E.M.	EDGAR MARCELINO CARVALHO	3098
CARVALHO, E.M.	EDGAR MARCELINO CARVALHO	3167
CARVALHO, E.M.	EDGAR MARCELINO CARVALHO	3192
CARVALHO, E.M.	EDGAR MARCELINO CARVALHO	3209
CARVALHO, E.M.	EDGAR MARCELINO CARVALHO	3370
CARVALHO, E.M.	EDGAR MARCELINO CARVALHO	3520
CARVALHO, F.R.	FABIANA RABE CARVALHO	2742
CARVALHO, F.R.	FABIANA RABE CARVALHO	2816
CARVALHO, F.R.	FABIANA RABE CARVALHO	2995
CARVALHO, H.C.D.O.F.	HELBER CARVALHO DE OLIVEIRA FERRAZ CARVALHO	3522
CARVALHO, J.G.	JÚLIA GALLO CARVALHO	2653
CARVALHO, J.G.	JÚLIA GALLO CARVALHO	2654
CARVALHO, K.A.B.	KELLY ALVES BICALHO CARVALHO	2667
CARVALHO, K.A.B.	KELLY ALVES BICALHO CARVALHO	3032
CARVALHO, L.D.A.	LETÍCIA DE ALMEIDA CARVALHO	3467
CARVALHO, L.G.	LUCAS GONCALVES CARVALHO	2901
CARVALHO, L.H.	LUZIA H. CARVALHO	2666
CARVALHO, M.	MÁRCIO CARVALHO	2952
CARVALHO, M.D.P.S.S.	MARIA DO PERPETUO SOCORRO SAMPAIO CARVALHO	3395
CARVALHO, M.F.	MARIANA FERNANDES CARVALHO	2814
CARVALHO, M.L.R.	MARIA LETICIA RODIGUES CARVALHO	2827
CARVALHO, M.L.R.	MARIA LETICIA RODIGUES CARVALHO	2841
CARVALHO, M.L.R.	MARIA LETICIA RODIGUES CARVALHO	3094
CARVALHO, M.L.R.	MARIA LETICIA RODIGUES CARVALHO	3171
CARVALHO, M.L.R.	MARIA LETICIA RODIGUES CARVALHO	3309
CARVALHO, M.L.R.	MARIA LETICIA RODRIGUES CARVALHO	3029
CARVALHO, N.O.	NANI OLIVEIRA CARVALHO	2635
CARVALHO, N.O.	NANI OLIVEIRA CARVALHO	2667
CARVALHO, W.A.	WANEISSA ARAUJO CARVALHO	2906
CARVALHO, W.A.	WANEISSA ARAUJO CARVALHO	3096
CARVALHO, W.A.	WANEISSA ARAUJO CARVALHO	3100
CARVALHO, W.A.	WANEISSA ARAUJO CARVALHO	3273
CARVALHO, W.A.	WANEISSA ARAUJO CARVALHO	3280

CARVALHO, W.A.	WANESSA ARAUJO CARVALHO	3450
CASAI, P.	PAULA CASAI	3508
CASARAVILLA, C.	CECILIA CASARAVILLA	2820
CASCABULHO, C.M.	CYNTHIA MACHADO CASCABULHO	3105
CASSALI, G.D.	GEOVANNI DANTAS CASSALI	2698
CASSALI, G.D.	GEOVANNI DANTAS CASSALI	2699
CASSALI, G.D.	GEOVANNI DANTAS CASSALI	2701
CASSALI, G.D.	GEOVANNI DANTAS CASSALI	2702
CASSEL, M.	MÔNICA CASSEL	2696
CASSIANO, A.C.M.	ANA CAROLINA MONTELEONE CASSIANO	3270
CASTANHEIRA, F.V.E.S.	FERNANDA VARGAS E SILVA CASTANHEIRA	2853
CASTANHOLA-DIAS,, L.	LUCAS CASTANHOLA-DIAS,	3326
CASTELO, A.P.	ADEMILSON PANUNTO CASTELO	3129
CASTELO-BRANCO, M.T.L.	MORGANA TEIXEIRA LIMA CASTELO-BRANCO	3070
CASTELO-BRANCO, M.T.L.	MORGANA TEIXEIRA LIMA CASTELO-BRANCO	3109
CASTELUCCI, B.G.	BIANCA GAZIERI CASTELUCCI	2772
CASTELUCCI, B.G.	BIANCA GAZIERI CASTELUCCI	2829
CASTELUCCI, B.G.	BIANCA GAZIERI CASTELUCCI	2863
CASTELUCCI, B.G.	BIANCA GAZIERI CASTELUCCI	2882
CASTELUCCI, B.G.	BIANCA GAZIERI CASTELUCCI	3034
CASTELUCCI, B.G.	BIANCA GAZIERI CASTELUCCI	3252
CASTELUCCI, B.G.	BIANCA GAZIERI CASTELUCCI	3487
CASTELUCCI, P.	PATRICIA CASTELUCCI	3076
CASTILHO, J.L.	JESSICA L. CASTILHO	3362
CASTILHO, L.	LEDA CASTILHO	2941
CASTILHO, L.	LEDA CASTILHO	2977
CASTILHO, L.	LEDA CASTILHO	3294
CASTINEIRAS, T.M.	TEREZINHA M. CASTINEIRAS	2719
CASTINEIRAS, T.M.	TEREZINHA M. CASTINEIRAS	3085
CASTIÑEIRAS, T.M.P.P.	TEREZINHA MARTA PEREIRA PINTO CASTIÑEIRAS	2818
CASTIÑEIRAS, T.M.P.P.	TEREZINHA MARTA PEREIRA PINTO CASTIÑEIRAS	2941
CASTIÑEIRAS, T.M.P.P.	TEREZINHA MARTA PEREIRA PINTO CASTIÑEIRAS	2963
CASTIÑEIRAS, T.M.P.P.	TEREZINHA MARTA PEREIRA PINTO CASTIÑEIRAS	2977
CASTRO, C.F.	CAMILA FERREIRA CASTRO	2952
CASTRO, G.	GISELE CASTRO	2809
CASTRO, G.	GISELE CASTRO	2832
CASTRO, G.	GISELE CASTRO	2863
CASTRO, J.M.D.A.	JUCIANE MARIA DE ANDRADE CASTRO	2834
CASTRO, J.M.D.A.	JUCIANE MARIA DE ANDRADE CASTRO	2979
CASTRO, J.M.D.A.	JUCIANE MARIA DE ANDRADE CASTRO	3352
CASTRO, L.L.M.E.	LUCIANA LISBOA MOTA E CASTRO	3294
CASTRO, M.D.S.	MARIANA DE SOUZA CASTRO	2839
CASTRO, M.J.C.	MAURO JORGE CABRAL CASTRO	2810
CASTRO, M.J.C.	MAURO JORGE CABRAL CASTRO	2816
CASTRO, M.J.C.	MAURO JORGE CABRAL CASTRO	2995
CASTRO, M.J.C.	MAURO JORGE CABRAL CASTRO	2998
CASTRO, M.J.C.	MAURO JORGE CABRAL CASTRO	3006
CASTRO, M.J.C.	MAURO JORGE CABRAL CASTRO	3136
CASTRO, M.S.	MARIANE SCHÄFFER CASTRO	3321
CASTRO, M.S.	MARIANE SCHÄFFER CASTRO	3435
CASTRO, M.V.D.S.	MARCUS VINICIUS DA SILVA CASTRO	3170
CASTRO, M.V.D.S.	MARCUS VINICIUS DA SILVA CASTRO	3415
CASTRO, R.	RICARDO CASTRO	2867
CASTRO, R.	RICARDO CASTRO	3018
CASTRO, V.P.E.	VIRGINIA PICANCO E CASTRO	2802
CATALDI, T.R.	THAÍS REGIANI CATALDI	2946
CATÃO, C.L.D.S.	CLAUDIO LUCAS DOS SANTOS CATÃO	3323
CATÃO, C.L.D.S.	CLAUDIO LUCAS DOS SANTOS CATÃO	3383
CATHARINO, L.C.C.	LUANA CORREIA CRODA CATHARINO	2762

CATHARINO, L.C.C.	LUANA CORREIA CRODA CATHARINO	3040
CATISSI, G.	GIULIA CATISSI	2777
CAVALCANTE, B.M.	BRENDA MARTINS CAVALCANTE	3026
CAVALCANTE, B.M.	BRENDA MARTINS CAVALCANTE	3028
CAVALCANTE, D.N.	DÉBORA NOGUEIRA CAVALCANTE	3414
CAVALCANTE, M.D.O.	MAYARA DE OLIVEIRA CAVALCANTE	3358
CAVALCANTE, M.D.S.	MAYSA DE SOUZA CAVALCANTE	2751
CAVALCANTE, M.D.S.	MAYSA DE SOUZA CAVALCANTE	2947
CAVALCANTE, S.D.F.	SAMANTHA DE FREITAS CAVALCANTE	3068
CAVALCANTE, S.D.F.	SAMANTHA DE FREITAS CAVALCANTE	3085
CAVALCANTE, T.T.A.	TÁVILA TATIANE AMORIM CAVALCANTE	2900
CAVALCANTE, T.T.A.	TÁVILA TATIANE AMORIM CAVALCANTE	3414
CAVALCANTI, E.J.O.	ELOÁ JARDIM OUTEIRO CAVALCANTI	3232
CAVALCANTI, E.J.O.	ELOÁ JARDIM OUTEIRO CAVALCANTI	3418
CAVAZZONI, C.B.	CECILIA BATAGLIOLI CAVAZZONI	2724
CAZOTE, A.D.S.	ANDRESSA DA SILVA CAZOTE	3000
CAZOTE, A.D.S.	ANDRESSA DA SILVA CAZOTE	3105
CAZOTE, A.D.S.	ANDRESSA DA SILVA CAZOTE	3240
CAZOTE, A.D.S.	ANDRESSA DA SILVA CAZOTE	3260
CEDRÉS, C.	CAMILA CEDRÉS	2951
CEDRO, M.M.	MARIANA MAY CEDRO	3508
CENEDEZE, M.A.	MARCOS ANTONIO CENEDEZE	3143
CERNA, G.	GABRIELLA CERNA	2783
CERNEA, C.R.	CLAUDIO ROBERTO CERNEA	3133
CERNI, F.A.	FELIPE AUGUSTO CERNI	3163
CERQUEIRA, M.M.O.P.	MÔNICA MARIA OLIVEIRA PINHO CERQUEIRA	3281
CHAGAS, A.C.D.S.	ANA CAROLINA DE SOUZA CHAGAS	2782
CHAGAS, G.G.	GIOVANA GOMES CHAGAS	3158
CHAGAS, G.G.	GIOVANA GOMES CHAGAS	3372
CHAGAS, J.D.A.	JULIANA DE ASSIS CHAGAS	2884
CHAGAS, J.D.A.	JULIANA DE ASSIS CHAGAS	3056
CHAGAS-NETO, A.J.	ALBERTO J. CHAGAS-NETO	3150
CHANASIT, S.	SUPAPICH CHANASIT	3182
CHAVES, A.N.	ALESSANDRA NUNES CHAVES	3435
CHAVES, A.P.C.	ANA PAULA CUNHA CHAVES	3042
CHAVES, L.B.	LANA BITENCOURT CHAVES	2743
CHAVES, L.B.	LANA BITENCOURT CHAVES	3355
CHAVES, M.M.	MARIANA M CHAVES	3465
CHAVES, T.C.	TALLES CAIRES CHAVES	3046
CHAVES, Y.O.	YURY OLIVEIRA CHAVES	3083
CHAVES, Y.O.	YURY OLIVEIRA CHAVES	3272
CHEN, S.	SIDI CHEN	2719
CHEVILLARD, C.	CHRISTOPHE CHEVILLARD	3074
CHIARI, G.C.S.	GABRIELLA CAROLINA SCALICE CHIARI	3415
CHIVALE, N.L.	NORDINO LUIS CHIVALE	2696
CHIVALE, N.L.	NORDINO LUIS CHIVALE	2697
CHIVALE, N.L.	NORDINO LUIS CHIVALE	2700
CHIVALE, N.L.	NORDINO LUIS CHIVALE	2703
CHIVALE, N.L.	NORDINO LUIS CHIVALE	2704
CHURA-CHAMBI, R.M.	ROSA M CHURA-CHAMBI	2858
CIANCAGLINI, P.	PIETRO CIANCAGLINI	3357
CINTRA, D.E.	DENNYS ESPER CINTRA	2869
CIONE, M.V.P.	MARCOS VINÍCIOS PINHEIRO CIONE	2989
CIONE, M.V.P.	MARCOS VINÍCIOS PINHEIRO CIONE	3008
CIONE, M.V.P.	MARCOS VINÍCIOS PINHEIRO CIONE	3143
CIPELLI, M.	MARCELLA CIPELLI	2784
CIPELLI, M.	MARCELLA CIPELLI	2917
CIPELLI, M.	MARCELLA CIPELLI	3143
CIPELLI, M.	MARCELLA CIPELLI	3348

CLARINDO, F.A.	FELIPE ALVES CLARINDO	2670
CLARINDO, F.A.	FELIPE ALVES CLARINDO	2691
CLARINDO, F.A.	FELIPE ALVES CLARINDO	2692
CLARINDO, F.A.	FELIPE ALVES CLARINDO	2693
CLARINDO, F.A.	FELIPE ALVES CLARINDO	3251
CLARKE, J.H.R.	JULIA HELENA ROSAURO CLARKE	3346
CLASER, C.	CARLA CLASER	3008
CLAUBERG, E.C.	EDUARDO CASA CLAUBERG	3288
CLEMENTE, B.T.	BRUNO TABELINI CLEMENTE	3421
CLEN, R.R.S.	ROSANA RODRIGUES SOARES CLEN	3405
CÓ, A.C.G.	ANNA CLARA GREGÓRIO CÓ	3409
COBOS, E.M.	ELENA MONTES COBOS	2795
COBOS, E.V.M.	ELENA VICTORIA MONTES COBOS	2941
COBOS, E.V.M.	ELENA VICTORIA MONTES COBOS	2963
COBOS, E.V.M.	ELENA VICTORIA MONTES COBOS	2977
COBOS, E.V.M.	ELENA VICTORIA MONTES COBOS	3086
COBOS, E.V.M.	ELENA VICTORIA MONTES COBOS	3145
CODOGNATO, S.G.	SOPHIA GRATON CODOGNATO	2989
CODOGNATO, S.G.	SOPHIA GRATON CODOGNATO	3008
CODOGNATO, S.G.	SOPHIA GRATON CODOGNATO	3325
COELHO, F.S.	FELIPE SOARES COELHO	3243
COELHO, F.S.	FELIPE SOARES COELHO	3249
COELHO, F.S.	FELIPE SOARES COELHO	3411
COELHO, G.R.	GUILHERME RABELO COELHO	3326
COELHO, J.	JULIA COELHO	3305
COELHO, J.	JULIA COELHO	3334
COELHO, L.F.L.	LUIZ FELIPE LEOMIL COELHO	3246
COELHO, M.G.P.	MARSEN GARCIA PINTO COELHO	2837
COELHO, M.G.P.	MARSEN GARCIA PINTO COELHO	3015
COELHO, M.G.P.	MARSEN GARCIA PINTO COELHO	3017
COELHO, M.G.P.	MARSEN GARCIA PINTO COELHO	3310
COELHO, M.M.	MONIQUE MACEDO COELHO	2643
COELHO, M.M.	MONIQUE MACEDO COELHO	2645
COELHO, M.M.	MONIQUE MACEDO COELHO	2664
COELHO, M.M.	MONIQUE MACEDO COELHO	2676
COELHO, M.M.	MONIQUE MACEDO COELHO	2679
COELHO, M.M.	MONIQUE MACEDO COELHO	2683
COELHO, M.P.	MÔNICA PEREIRA COELHO	3362
COELHO, M.P.	MÔNICA PEREIRA COELHO	3476
COELHO, M.P.S.	MARCOS PAULO SANTOS COELHO	2734
COELHO, M.P.S.	MARCOS PAULO SANTOS COELHO	2741
COELHO, M.P.S.	MARCOS PAULO SANTOS COELHO	2878
COELHO, M.P.S.	MARCOS PAULO SANTOS COELHO	2889
COELHO, M.P.S.	MARCOS PAULO SANTOS COELHO	2938
COELHO, M.P.S.	MARCOS PAULO SANTOS COELHO	2966
COELHO, M.P.S.	MARCOS PAULO SANTOS COELHO	2967
COELHO, M.P.S.	MARCOS PAULO SANTOS COELHO	3076
COELHO, P.M.P.	PEDRO MORENO PIMENTEL COELHO	3502
COELHO, S.V.A.	SHARTON VINICIUS ANTUNES COELHO	2898
COELHO, S.V.A.	SHARTON VINICIUS ANTUNES COELHO	2955
COELHO, S.V.A.	SHARTON VINICIUS ANTUNES COELHO	3041
COELHO, S.V.A.	SHARTON VINICIUS ANTUNES COELHO	3071
COELHO, S.V.A.	SHARTON VINICIUS ANTUNES COELHO	3239
COELHO-E-SILVA, M.J.	MANUEL J COELHO-E-SILVA	2866
COLAÇO, M.V.	MARCOS VINICIUS COLAÇO	2901
COLES, M.	MARK COLES	2862
COLETA, M.V.D.	MARCUS VINICIUS DELLA COLETA	3323
COLLI, B.	BRUNA COLLI	3065
COLODET, L.C.	LILIAN CASARTELLI COLODET	3494

COLOMBINI, M.	MONICA COLOMBINI	3225
COLOMBO, A.P.V.	ANA PAULA VIEIRA COLOMBO	3306
COLOMBO, F.A.	FÁBIO ANTÔNIO COLOMBO	3246
COLONNA, M.	MARCO COLONNA	2843
COLUMNNA, F.C.	FABIANE CRISTINA COLUMNNA	2945
COLUMNNA, F.C.	FABIANE CRISTINA COLUMNNA	3292
CONCEIÇÃO, M.L.P.	MICAELY LORRANA PEREIRA CONCEIÇÃO	3170
CONCEIÇÃO, M.L.P.	MICAELY LORRANA PEREIRA CONCEIÇÃO	3333
CONNELLEY, T.	TIMOTHY CONNELLEY	3033
CONSONNI, S.R.	SÍLVIO ROBERTO CONSONNI	2882
CONSTANCIO, C.D.S.	CLARICE DE SOUZA CONSTANCIO	2795
CONSTANCIO, C.D.S.	CLARICE DE SOUZA CONSTANCIO	2963
CONSTANCIO, C.D.S.	CLARICE DE SOUZA CONSTANCIO	3145
CONTE, F.D.P.	FERNANDO DE PAIVA CONTE	2743
CONTIN, S.V.M.	SARAH VIEIRA MARQUES CONTIN	2756
CONTIN, S.V.M.	SARAH VIEIRA MARQUES CONTIN	3267
CONTIN, S.V.M.	SARAH VIEIRA MARQUES CONTIN	3294
CONTINENTINO, A.L.	ALICE LIMA CONTINENTINO	3311
CORDEIRO, A.D.F.	AMANDA DE FRANÇA CORDEIRO	3055
CORDEIRO, A.D.F.	AMANDA DE FRANÇA CORDEIRO	3308
CORDEIRO, A.D.F.	AMANDA DE FRANÇA CORDEIRO	3310
CORDEIRO, A.D.F.	AMANDA DE FRANÇA CORDEIRO	3361
CORDEIRO, A.P.	ARTHUR POESTER CORDEIRO	3499
CORDEIRO, L.P.B.	LÍDIA PEREIRA BARBOSA CORDEIRO	2632
CORDEIRO, L.P.B.	LÍDIA PEREIRA BARBOSA CORDEIRO	2633
CORDEIRO, L.P.B.	LÍDIA PEREIRA BARBOSA CORDEIRO	3509
CORDEIRO, R.	RITA CORDEIRO	2719
CORONA, L.M.	LUCAS MIRANDA CORONA	2879
CORRADI, P.S.	PEDRO SÉRGIO CORRADI	3305
CORRADI, P.S.	PEDRO SÉRGIO CORRADI	3334
CORRADI, P.S.	PEDRO SÉRGIO CORRADI	3465
CORREA, A.A.D.S.	ANDRÉ AP DOS SANTOS CORREA	2914
CORREA, A.A.D.S.	ANDRÉ AP DOS SANTOS CORREA	3513
CORREA, A.M.C.	ARYELLA MARYAH COUTO CORREA	3036
CORRÊA, B.	BRUNO CORRÊA	3289
CORRÊA, B.	BRUNO CORRÊA	3488
CORREA, F.C.	FERNANDO COUSO CORREA	3382
CORREA, F.M.D.A.	FERNANDO MORGAN DE AGUIAR CORREA	3269
CORREA, G.A.D.C.	GIULIANA ABREU DA COSTA CORREA	3343
CORRÊA, L.G.P.	LORRANE GABRIELE PINHEIRO CORRÊA	2782
CORRÊA, M.E.H.	MARIA EUGÊNIA HOTT CORRÊA	3051
CORRÊA, P.R.L.	PAULO ROBERTO LOPES CORRÊA	2756
CORRÊA, P.R.L.	PAULO ROBERTO LOPES CORRÊA	3267
CORREA, R.	RAFAEL CORREA	3001
CORREIA, E.M.	EDUARDO MANNARINO CORREIA	3029
CORREIA, E.M.	EDUARDO MANNARINO CORREIA	3108
CORREIA, E.M.	EDUARDO MANNARINO CORREIA	3153
CORREIA, E.M.	EDUARDO MANNARINO CORREIA	3171
CORREIA, E.M.	EDUARDO MANNARINO CORREIA	3385
CORREIA, E.M.	EDUARDO MANNARINO CORREIA	3443
CORREIA, I.P.R.	IVANA PATRICIA RIBEIRO CORREIA	3373
CORREIA, I.P.R.	IVANA PATRICIA RIBEIRO CORREIA	3406
CORREIA, I.P.R.	IVANA PATRICIA RIBEIRO CORREIA	3441
CORREIA, I.P.R.	IVANA PATRICIA RIBEIRO CORREIA	3504
CORSETTI, P.P.	PATRÍCIA PAIVA CORSETTI	2962
CORSETTI, P.P.	PATRÍCIA PAIVA CORSETTI	2965
CORSETTI, P.P.	PATRÍCIA PAIVA CORSETTI	3003
CORSINI, C.A.	CAMILA AMORMINO CORSINI	3294
CÔRTEZ, F.H.	FERNANDA HELOISE CÔRTEZ	3000

CÔRTEZ, F.H.	FERNANDA HELOISE CÔRTEZ	3064
CÔRTEZ, F.H.	FERNANDA HELOISE CÔRTEZ	3105
CÔRTEZ, F.H.	FERNANDA HELOISE CÔRTEZ	3240
CÔRTEZ, F.H.	FERNANDA HELOISE CÔRTEZ	3260
CORTES, L.G.F.	LUIZ GUSTAVO FERREIRA CORTES	3060
CORTES, L.G.F.	LUIZ GUSTAVO FERREIRA CORTES	3293
CORTES, L.G.F.	LUIZ GUSTAVO FERREIRA CORTES	3314
CORTES, L.G.F.	LUIZ GUSTAVO FERREIRA CORTES	3364
CÔRTEZ, T.N.	THAÍS NASCIMENTO CÔRTEZ	3306
COSENTINO-GOMES, D.	DANIELA COSENTINO-GOMES	2936
COSTA, A.G.B.	ANDERSON GUIMARÃES BAPTISTA COSTA	2930
COSTA, A.G.B.	ANDERSON GUIMARÃES BAPTISTA COSTA	2955
COSTA, A.G.B.	ANDERSON GUIMARÃES BAPTISTA COSTA	3041
COSTA, A.G.B.	ANDERSON GUIMARÃES BAPTISTA COSTA	3071
COSTA, A.G.B.	ANDERSON GUIMARÃES BAPTISTA COSTA	3106
COSTA, A.G.B.	ANDERSON GUIMARÃES BAPTISTA COSTA	3239
COSTA, A.G.B.	ANDERSON GUIMARÃES BAPTISTA COSTA	3473
COSTA, A.L.M.D.S.	ANA LUIZA MAGOCO DA SILVA COSTA	3460
COSTA, C.M.	CAMILA MEDEIROS COSTA	2636
COSTA, C.M.	CAMILA MEDEIROS COSTA	2639
COSTA, C.M.	CAMILA MEDEIROS COSTA	2650
COSTA, D.D.	DÉBORA DIAS COSTA	2891
COSTA, D.L.	DIEGO LUIS COSTA	2914
COSTA, D.L.	DIEGO LUIS COSTA	2997
COSTA, D.L.	DIEGO LUIS COSTA	3158
COSTA, D.L.	DIEGO LUIS COSTA	3513
COSTA, E.I.F.S.	EMILE IVANA FERNANDES SANTOS COSTA	3312
COSTA, F.F.	FERNANDO FERREIRA COSTA	2845
COSTA, F.F.	FERNANDO FERREIRA COSTA	2848
COSTA, G.M.J.	GUILHERME M J COSTA	2685
COSTA, J.C.	JÚLIA CURVELO COSTA	3046
COSTA, J.S.R.	JULIANA SALES RODRIGUES COSTA	2987
COSTA, L.	LUCIANA COSTA	3041
COSTA, M.A.	MARINA AMORIM COSTA	2709
COSTA, M.A.	MARINA AMORIM COSTA	2718
COSTA, M.C.D.S.	MARCELA CASTRO DOS SANTOS COSTA	3366
COSTA, M.G.	MAURICIO GARCIA COSTA	3174
COSTA, M.G.	MAURICIO GARCIA COSTA	3371
COSTA, M.L.B.	MARINA LUIZA BAÊTA COSTA	2987
COSTA, M.L.B.	MARINA LUIZA BAÊTA COSTA	3165
COSTA, M.S.	MURILO SOARES COSTA	2643
COSTA, M.S.	MURILO SOARES COSTA	2645
COSTA, R.D.S.	RYAN DOS SANTOS COSTA	3312
COSTA, R.S.	RENATA SESTI COSTA	2845
COSTA, R.S.	RENATA SESTI COSTA	2848
COSTA, R.S.	RUBIA SUELY COSTA	2921
COSTA, R.S.	RUBIA SUELY COSTA	3441
COSTA, R.S.	RUBIA SUELY COSTA	3504
COSTA, T.D.S.	TAYLLA DOS SANTOS COSTA	2779
COSTA, T.D.S.	TAYLLA DOS SANTOS COSTA	3210
COSTA, V.F.	VICTOR FERREIRA COSTA	3408
COSTA, V.V.	VIVIAN VASCONCELOS COSTA	2685
COSTA, V.V.	VIVIAN VASCONCELOS COSTA	2686
COSTA, V.V.	VIVIAN VASCONCELOS COSTA	2699
COSTA, V.V.	VIVIAN VASCONCELOS COSTA	2701
COSTA, V.V.	VIVIAN VASCONCELOS COSTA	2988
COSTA, V.V.	VIVIAN VASCONCELOS COSTA	3130
COSTA, V.V.	VIVIAN VASCONCELOS COSTA	3185
COSTA, V.V.	VIVIAN VASCONCELOS COSTA	3316

COSTA, V.V.	VIVIAN VASCONCELOS COSTA	3421
COSTABEL, M.	MARCELO COSTABEL	2957
COTTA-DE-ALMEIDA, V.	VINICIUS COTTA-DE-ALMEIDA	3052
COUILLIN, I.	ISABELLE COUILLIN	3337
COUNSELL, J.	JOHN COUNSELL	2844
COURROL, D.D.S.	DANIELLA DOS SANTOS COURROL	2858
COURROL, D.D.S.	DANIELLA DOS SANTOS COURROL	2990
COURROL, D.D.S.	DANIELLA DOS SANTOS COURROL	3020
COUTINHO, V.M.	VINICIUS MARACAJA COUTINHO	2724
COUTINHO-SILVA, R.	ROBSON COUTINHO-SILVA	3008
COUTINHO-SILVA, R.	ROBSON COUTINHO-SILVA	3230
COUTINHO-SILVA, R.	ROBSON COUTINHO-SILVA	3306
COUTINHO-SILVA, R.	ROBSON COUTINHO-SILVA	3393
COUTINHO-SILVA, R.	ROBSON COUTINHO-SILVA	3467
COUTINHO-SILVA, R.	ROBSON COUTINHO-SILVA	3491
COUTINHO-SILVA, R.	ROBSON COUTINHO-SILVA	3494
COUTO, D.S.	DIOGO SENA COUTO	3265
COUTO, S.C.F.	SAMUEL CAMPANELLI FREITAS COUTO	2854
COUTO, S.C.F.	SAMUEL CAMPANELLI FREITAS COUTO	2855
COUTO, S.C.F.	SAMUEL CAMPANELLI FREITAS COUTO	3221
COUTO, S.C.F.	SAMUEL CAMPANELLI FREITAS COUTO	3226
COUTO, S.C.F.	SAMUEL CAMPANELLI FREITAS COUTO	3236
COUTO, S.C.F.	SAMUEL CAMPANELLI FREITAS COUTO	3410
COVAS, D.T.	DIMAS TADEU COVAS	3016
COVRE, L.P.	LUCIANA POLACO COVRE	2787
COVRE, L.P.	LUCIANA POLACO COVRE	2862
COVRE, L.P.	LUCIANA POLACO COVRE	2868
COVRE, L.P.	LUCIANA POLACO COVRE	2898
COVRE, L.P.	LUCIANA POLACO COVRE	2980
COVRE, L.P.	LUCIANA POLACO COVRE	3059
COVRE, L.P.	LUCIANA POLACO COVRE	3123
COVRE, L.P.	LUCIANA POLACO COVRE	3227
COVRE, L.P.	LUCIANA POLACO COVRE	3232
COVRE, L.P.	LUCIANA POLACO COVRE	3403
COVRE, L.P.	LUCIANA POLACO COVRE	3409
COVRE, L.P.	LUCIANA POLACO COVRE	3418
COVRE, L.P.	LUCIANA POLACO COVRE	3483
COVRE, L.P.	LUCIANA POLACO COVRE	3484
COVRE, L.P.	LUCIANA POLACO COVRE	3489
CRANDELL, J.	JAMESON CRANDELL	2719
CREPALDI, L.D.	LETICIA DINIZ CREPALDI	3494
CRISTAL, J.R.S.	JUQUELINE ROCHA SANTOS CRISTAL	3075
CRISTAL, J.R.S.	JUQUELINE ROCHA SANTOS CRISTAL	3520
CRISTOVAM, P.C.	PRISCILA CARDOSO CRISTOVAM	2759
CRODA, J.H.R.	JULIO HENRIQUE ROSA CRODA	3154
CRUZ, A.K.	ANGELA KAYSEL CRUZ	3465
CRUZ, C.C.O.D.M.	CAROLINA CALÔBA OLIVEIRA DE MATTOS CRUZ	3394
CRUZ, E.F.	EDGAR FERREIRA CRUZ	2939
CRUZ, J.D.S.	JADER DOS SANTOS CRUZ	2641
CRUZ, J.D.S.	JADER DOS SANTOS CRUZ	2699
CRUZ, L.F.	LUAN FIRMINO CRUZ	3484
CRUZ, L.O.R.	LETICIA OLIVEIRA ROJAS CRUZ	3124
CRUZ, M.C.	MÁRIO COSTA CRUZ	2792
CRUZ, M.C.	MÁRIO COSTA CRUZ	3230
CRUZ, P.C.O.D.M.	PHILIPPE CALOBA OLIVEIRA DE MATTOS CRUZ	2724
CRUZ, P.C.O.D.M.	PHILIPPE CALOBA OLIVEIRA DE MATTOS CRUZ	3394
CRUZ, P.E.D.O.	PAULO EMILIO DE OLIVEIRA CRUZ	3182
CRUZ, P.E.D.O.	PAULO EMILIO DE OLIVEIRA CRUZ	3366
CRUZ, R.G.B.	RODRIGO GOMES BERNARDO CRUZ	2733

CUELLAR, R.A.D.	ROSA AMALIA DUEÑAS CUELLAR	2725
CUNHA, D.P.	DANIELA PRADO CUNHA	3174
CUNHA, D.P.	DANIELA PRADO CUNHA	3184
CUNHA, D.P.	DANIELA PRADO CUNHA	3243
CUNHA, D.P.	DANIELA PRADO CUNHA	3268
CUNHA, D.P.	DANIELA PRADO CUNHA	3371
CUNHA, F.Q.	FERNANDO Q CUNHA	2750
CUNHA, F.Q.	FERNANDO Q CUNHA	2755
CUNHA, F.Q.	FERNANDO Q CUNHA	2852
CUNHA, F.Q.	FERNANDO Q CUNHA	3022
CUNHA, F.Q.	FERNANDO Q CUNHA	3054
CUNHA, F.Q.	FERNANDO Q CUNHA	3088
CUNHA, F.Q.	FERNANDO Q CUNHA	3097
CUNHA, F.Q.	FERNANDO Q CUNHA	3160
CUNHA, F.Q.	FERNANDO Q CUNHA	3168
CUNHA, F.Q.	FERNANDO Q CUNHA	3170
CUNHA, F.Q.	FERNANDO Q CUNHA	3206
CUNHA, F.Q.	FERNANDO Q CUNHA	3269
CUNHA, F.Q.	FERNANDO Q CUNHA	3333
CUNHA, F.Q.	FERNANDO Q CUNHA	3408
CUNHA, F.Q.	FERNANDO Q CUNHA	3415
CUNHA, L.C.	LAURA COLETE CUNHA	3307
CUNHA, L.D.	LARISSA D CUNHA	2772
CUNHA, L.D.	LARISSA D CUNHA	2808
CUNHA, L.D.	LARISSA D CUNHA	3084
CUNHA, L.D.	LARISSA D CUNHA	3128
CUNHA, L.D.	LARISSA D CUNHA	3224
CUNHA, L.D.	LARISSA D CUNHA	3271
CUNHA, L.D.	LARISSA D CUNHA	3367
CUNHA, L.D.	LARISSA D CUNHA	3492
CUNHA, T.M.	THIAGO MATTAR CUNHA	2750
CUNHA, T.M.	THIAGO MATTAR CUNHA	2755
CUNHA, T.M.	THIAGO MATTAR CUNHA	2833
CUNHA, T.M.	THIAGO MATTAR CUNHA	2852
CUNHA, T.M.	THIAGO MATTAR CUNHA	3022
CUNHA, T.M.	THIAGO MATTAR CUNHA	3054
CUNHA, T.M.	THIAGO MATTAR CUNHA	3088
CUNHA, T.M.	THIAGO MATTAR CUNHA	3097
CUNHA, T.M.	THIAGO MATTAR CUNHA	3170
CUNHA, T.M.	THIAGO MATTAR CUNHA	3269
CUNHA, T.M.	THIAGO MATTAR CUNHA	3333
CUNHA, T.M.	THIAGO MATTAR CUNHA	3369
CUNHA, T.M.	THIAGO MATTAR CUNHA	3376
CUNHA, T.M.	THIAGO MATTAR CUNHA	3415
CUNHA-NETO, E.	EDÉCIO CUNHA-NETO	2740
CUNHA-NETO, E.	EDÉCIO CUNHA-NETO	3074
CUNHA-NETO, E.	EDÉCIO CUNHA-NETO	3149
CURI, R.	RUI CURI	2879
CURI, R.	RUI CURI	2902
CYPRESTE, L.T.	LARA TAVARES CYPRESTE	3046
CZYZ, S.	SONIA CZYZ	2926
DA CONCEIÇÃO JUNIOR, F.	FLÁVIO DA CONCEIÇÃO JUNIOR	2905
DA CONCEIÇÃO, G.R.	GIOVANNA REIS DA CONCEIÇÃO	2656
DA CONCEIÇÃO, G.R.	GIOVANNA REIS DA CONCEIÇÃO	2665
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2734
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2735
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2741
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2878
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2889

DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2938
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2944
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2966
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2967
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	2982
DA CONCEIÇÃO, L.B.	LAURA BARBOSA DA CONCEIÇÃO	3076
DA COSTA, A.A.B.A.	ALEXANDRE ANDRE BALIEIRO ANASTACIO DA COSTA	2827
DA COSTA, A.C.C.	ANA CAROLINA CARIOCA DA COSTA	3187
DA COSTA, A.G.	ALLYSON GUIMARÃES DA COSTA	3323
DA COSTA, A.G.	ALLYSON GUIMARÃES DA COSTA	3383
DA COSTA, A.G.	ALLYSON GUIMARÃES DA COSTA	3395
DA COSTA, A.G.	ALLYSON GUIMARÃES DA COSTA	3414
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	2897
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	2904
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3099
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3132
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3138
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3195
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3238
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3241
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3256
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3261
DA COSTA, A.L.A.	ANDRE LUIZ AMORIM DA COSTA	3306
DA COSTA, A.R.	ANDRÉA RODRIGUES DA COSTA	3359
DA COSTA, E.C.	ESTEVÃO CAMILLO DA COSTA	2782
DA COSTA, E.V.	ESTHER VENTURA DA COSTA	2758
DA COSTA, F.P.	FILIPE PEREIRA DA COSTA	3359
DA COSTA, H.H.M.	HERNAN HERMES MONTEIRO DA COSTA	3002
DA COSTA, H.H.M.	HERNAN HERMES MONTEIRO DA COSTA	3042
DA COSTA, H.H.M.	HERNAN HERMES MONTEIRO DA COSTA	3450
DA COSTA, L.B.	LAÍS BEZERRA DA COSTA	3069
DA COSTA, L.M.P.	LUÍSA MEIRELES PESSOA DA COSTA	2716
DA COSTA, R.G.	RAFAEL GARRETT DA COSTA	3404
DA COSTA, S.P.	SANDRO PINHEIRO DA COSTA	3471
DA COSTA, T.L.	THAIS LIMA DA COSTA	2968
DA COSTA, W.L.G.	WEBSTER LEONARDO GUIMARÃES DA COSTA	2809
DA COSTA, W.L.G.	WEBSTER LEONARDO GUIMARÃES DA COSTA	2831
DA COSTA, W.L.G.	WEBSTER LEONARDO GUIMARÃES DA COSTA	2863
DA COSTA, W.L.G.	WEBSTER LEONARDO GUIMARÃES DA COSTA	3004
DA COSTA, W.L.G.	WEBSTER LEONARDO GUIMARÃES DA COSTA	3365
DA CRUZ, D.A.M.	DANIELLA ARÊAS MENDES DA CRUZ	3151
DA CRUZ, E.D.D.S.F.	EDUARDA DIAS DA SILVA FERREIRA DA CRUZ	3468
DA CRUZ, G.I.P.	GUILHERME IACK PINHEIRO DA CRUZ	3257
DA CRUZ, G.I.P.	GUILHERME IACK PINHEIRO DA CRUZ	3429
DA CRUZ, G.I.P.	GUILHERME IACK PINHEIRO DA CRUZ	3439
DA CRUZ, L.D.O.	LORRAINE DE OLIVEIRA DA CRUZ	2904
DA CRUZ, L.D.O.	LORRAINE DE OLIVEIRA DA CRUZ	3306
DA CRUZ, L.D.O.	LORRAINE DE OLIVEIRA DA CRUZ	3393
DA CRUZ, L.F.M.	LUÍS FELIPE MARTINS DA CRUZ	2706
DA CRUZ, L.L.P.	LORRAYNNE LETYCIA PRADO DA CRUZ	2895
DA CUNHA, A.F.	AMANDA FREITAS DA CUNHA	2782
DA CUNHA, F.N.	FERNANDA NEVES DA CUNHA	3014
DA CUNHA, F.N.	FERNANDA NEVES DA CUNHA	3274
DA FONSECA, D.L.M.	DENNYSON LEANDRO MATHIAS DA FONSECA	2889
DA FONSECA, D.L.M.	DENNYSON LEANDRO MATHIAS DA FONSECA	3008
DA FONSECA, D.L.M.	DENNYSON LEANDRO MATHIAS DA FONSECA	3124
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2645
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2664
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2673

DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2734
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2735
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2741
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2801
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2811
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2878
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2889
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2938
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2944
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2966
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2967
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	2982
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	3067
DA FONSECA, D.M.	DENISE MORAIS DA FONSECA	3076
DA FONSECA, F.G.	FLÁVIO GUIMARÃES DA FONSECA	2674
DA FONSECA, F.G.	FLÁVIO GUIMARÃES DA FONSECA	2687
DA FONSECA, F.G.	FLÁVIO GUIMARÃES DA FONSECA	2690
DA FONSECA, F.G.	FLÁVIO GUIMARÃES DA FONSECA	2691
DA FONSECA, F.G.	FLÁVIO GUIMARÃES DA FONSECA	2692
DA FONSECA, F.G.	FLÁVIO GUIMARÃES DA FONSECA	2693
DA FONSECA, F.G.	FLÁVIO GUIMARÃES DA FONSECA	2714
DA FONSECA, F.G.	FLÁVIO GUIMARÃES DA FONSECA	3281
DA FONSECA, L.M.	LEONARDO MARQUES DA FONSECA	2738
DA FONSECA, P.B.	PEDRO BARBOSA DA FONSECA	2810
DA FONSECA, P.B.	PEDRO BARBOSA DA FONSECA	3006
DA FONSECA, T.C.M.	TALITA CRISTINA MARTINS DA FONSECA	2686
DA FONSECA, T.C.M.	TALITA CRISTINA MARTINS DA FONSECA	2988
DA FONTOURA, J.C.	JÚLIA CRISPIM DA FONTOURA	3353
DA GRAÇA-FILHO, R.V.	RENAN VASCONCELOS DA GRAÇA-FILHO	2675
DA GRAÇA-FILHO, R.V.	RENAN VASCONCELOS DA GRAÇA-FILHO	3080
DA LUZ, C.S.	CAROLINE SIMÕES DA LUZ	3055
DA LUZ, C.S.	CAROLINE SIMÕES DA LUZ	3308
DA LUZ, C.S.	CAROLINE SIMÕES DA LUZ	3361
DA MATA, C.P.S.M.	CAMILA PACHECO SILVEIRA MARTINS DA MATA	2691
DA POIAN, A.T.	ANDREA THOMPSON DA POIAN	3304
DA POIAN, A.T.	ANDREA THOMPSON DA POIAN	3417
DA ROCHA, A.A.	ALISSON AMARAL DA ROCHA	3343
DA ROCHA, E.L.	EDROALDO LUMMERTZ DA ROCHA	3288
DA ROCHA, J.J.R.	JOSÉ JOAQUIM RIBEIRO DA ROCHA	3381
DA ROSA, S.A.	OLON ANDRADES DA ROSA	3353
DA SILVA JUNIOR, E.B.	ELIAS BARBOSA DA SILVA JUNIOR	2885
DA SILVA JUNIOR, E.B.	ELIAS BARBOSA DA SILVA JUNIOR	2912
DA SILVA JUNIOR, E.B.	ELIAS BARBOSA DA SILVA JUNIOR	2948
DA SILVA JUNIOR, E.B.	ELIAS BARBOSA DA SILVA JUNIOR	3484
DA SILVA JUNIOR, P.I.	PEDRO ISMAEL DA SILVA JUNIOR	3358
DA SILVA, A.B.	ANELISE BAPTISTA DA SILVA	3289
DA SILVA, A.B.	ANELISE BAPTISTA DA SILVA	3488
DA SILVA, A.B.C.	ANNA BEATRIZ COSTA DA SILVA	3269
DA SILVA, A.B.C.	ANNA BEATRIZ COSTA DA SILVA	3320
DA SILVA, A.B.R.	ARTUR BARREIRAS RIBEIRO DA SILVA	2813
DA SILVA, A.B.R.	ARTUR BARREIRAS RIBEIRO DA SILVA	2822
DA SILVA, A.B.R.	ARTUR BARREIRAS RIBEIRO DA SILVA	3116
DA SILVA, A.C.B.	ANA CRISTINA BORGES DA SILVA	3521
DA SILVA, A.C.R.	ANA CAROLINA RODRIGUES DA SILVA	3519
DA SILVA, A.D.	ADILSON DAVID DA SILVA	3082
DA SILVA, A.D.	ADILSON DAVID DA SILVA	3102
DA SILVA, A.D.	ADILSON DAVID DA SILVA	3147
DA SILVA, A.D.	ADILSON DAVID DA SILVA	3162
DA SILVA, A.D.	ADILSON DAVID DA SILVA	3203

DA SILVA, A.F.	ALAN FRAZÃO DA SILVA	2811
DA SILVA, A.F.A.	AUGUSTO FILIPE ALMEIDA DA SILVA	2953
DA SILVA, A.J.	ADILSON JOSÉ DA SILVA	2819
DA SILVA, A.M.	ARISTÓBOLO MENDES DA SILVA	3421
DA SILVA, A.M.G.	ANA MARIA GONÇALVES DA SILVA	2792
DA SILVA, A.M.V.	ANDREA MARQUES VIEIRA DA SILVA	3249
DA SILVA, A.M.V.	ANDRÉA MARQUES VIEIRA DA SILVA	3243
DA SILVA, A.M.V.	ANDRÉA MARQUES VIEIRA DA SILVA	3411
DA SILVA, A.P.S.	ANA PAULA SOARES DA SILVA	2879
DA SILVA, A.Q.P.	ALANNA QUÉSEDE PAIVA DA SILVA	3083
DA SILVA, A.Q.P.	ALANNA QUÉSEDE PAIVA DA SILVA	3272
DA SILVA, A.S.R.	ADELINO SANCHEZ RAMOS DA SILVA	2869
DA SILVA, C.H.	CLEBER HOOPER DA SILVA	3501
DA SILVA, C.P.	CAROLINA PEREIRA DA SILVA	2971
DA SILVA, C.P.	CAROLINA PEREIRA DA SILVA	2983
DA SILVA, C.V.	CLAUDIO VIEIRA DA SILVA	2695
DA SILVA, C.V.	CLAUDIO VIEIRA DA SILVA	2696
DA SILVA, C.V.	CLAUDIO VIEIRA DA SILVA	2697
DA SILVA, C.V.	CLAUDIO VIEIRA DA SILVA	2700
DA SILVA, C.V.	CLAUDIO VIEIRA DA SILVA	2703
DA SILVA, C.V.	CLAUDIO VIEIRA DA SILVA	2704
DA SILVA, D.P.	DAIANA PERCE DA SILVA	3355
DA SILVA, E.L.	ELSON LONGO DA SILVA	3255
DA SILVA, E.M.	ELOISA MARTINS DA SILVA	2784
DA SILVA, E.M.	ELOISA MARTINS DA SILVA	2917
DA SILVA, E.P.D.	EDUARDO PEREIRA DUARTE DA SILVA	3097
DA SILVA, E.R.	EDUARDA RODRIGUES DA SILVA	3253
DA SILVA, E.S.	EDUARDO SANTOS DA SILVA	2850
DA SILVA, E.S.	EDUARDO SANTOS DA SILVA	3182
DA SILVA, E.S.	EDUARDO SANTOS DA SILVA	3197
DA SILVA, E.S.	EDUARDO SANTOS DA SILVA	3366
DA SILVA, F.R.	FLÁVIO ROCHA DA SILVA	3047
DA SILVA, F.S.	FLAVIO SOUZA DA SILVA	3395
DA SILVA, G.B.L.	GABRIELLE BRUM LOPES DA SILVA	3394
DA SILVA, G.C.P.	GEAN CARLO PEREIRA DA SILVA	2898
DA SILVA, G.C.P.	GEAN CARLO PEREIRA DA SILVA	2905
DA SILVA, G.C.P.	GEAN CARLO PEREIRA DA SILVA	3049
DA SILVA, G.C.P.	GEAN CARLO PEREIRA DA SILVA	3304
DA SILVA, G.D.	GABRIELA DUARTE DA SILVA	2775
DA SILVA, G.D.	GABRIELA DUARTE DA SILVA	3156
DA SILVA, G.D.	GABRIELA DUARTE DA SILVA	3250
DA SILVA, G.D.	GABRIELA DUARTE DA SILVA	3298
DA SILVA, G.D.	GABRIELA DUARTE DA SILVA	3464
DA SILVA, G.M.	GABRIELA MACIEL DA SILVA	2719
DA SILVA, G.M.	GABRIELA MACIEL DA SILVA	3478
DA SILVA, G.R.	GUILHERME RIBEIRO DA SILVA	2772
DA SILVA, G.R.	GUILHERME RIBEIRO DA SILVA	2829
DA SILVA, G.R.	GUILHERME RIBEIRO DA SILVA	2832
DA SILVA, G.R.	GUILHERME RIBEIRO DA SILVA	2882
DA SILVA, G.R.	GUILHERME RIBEIRO DA SILVA	2920
DA SILVA, G.R.	GUILHERME RIBEIRO DA SILVA	3027
DA SILVA, G.R.	GUILHERME RIBEIRO DA SILVA	3034
DA SILVA, G.R.	GUILHERME RIBEIRO DA SILVA	3487
DA SILVA, G.V.L.	GABRIEL VICTOR LUCENA DA SILVA	3333
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2673
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2734
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2735
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2741
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2801

DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2878
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2889
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2938
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2944
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2966
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2967
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	2982
DA SILVA, G.W.	GUILHERME WILLIAM DA SILVA	3076
DA SILVA, H.B.	HENRIQUE BORGES DA SILVA	2935
DA SILVA, I.B.	IRIS BRAGA DA SILVA	2816
DA SILVA, I.B.	IRIS BRAGA DA SILVA	3245
DA SILVA, I.B.S.	ICARO BONYEK SANTOS DA SILVA	3125
DA SILVA, I.B.S.	ICARO BONYEK SANTOS DA SILVA	3156
DA SILVA, I.B.S.	ICARO BONYEK SANTOS DA SILVA	3318
DA SILVA, I.M.	IGOR MARTINS DA SILVA	2826
DA SILVA, I.T.	ISRAEL TOJAL DA SILVA	3314
DA SILVA, I.T.	ISRAEL TOJAL DA SILVA	3364
DA SILVA, J.A.	JULIANA AZEVEDO DA SILVA	3377
DA SILVA, J.A.	JULIANA AZEVEDO DA SILVA	3405
DA SILVA, J.B.D.P.	JÚLIA BARLETA DE PAULA DA SILVA	2815
DA SILVA, J.F.A.	JULIANA FERNANDES AMORIM DA SILVA	2812
DA SILVA, J.O.	JEFFERSON OLIVEIRA DA SILVA	3326
DA SILVA, J.O.	JULLYANNA OLIVEIRA DA SILVA	2955
DA SILVA, J.O.	JULLYANNA OLIVEIRA DA SILVA	3041
DA SILVA, J.O.	JULLYANNA OLIVEIRA DA SILVA	3071
DA SILVA, J.O.	JULLYANNA OLIVEIRA DA SILVA	3473
DA SILVA, J.R.	JAMILE RAMOS DA SILVA	3472
DA SILVA, J.S.	JULIA SANTOS DA SILVA	2919
DA SILVA, J.S.	JULIA SANTOS DA SILVA	3328
DA SILVA, J.V.E.	JOÃO VITOR ELEUTERIO DA SILVA	2707
DA SILVA, J.V.H.	JOÃO VINÍCIUS HONÓRIO DA SILVA	2917
DA SILVA, J.V.V.	JOÃO VÍCTOR VIRGÍLIO DA SILVA	2882
DA SILVA, J.V.V.	JOÃO VÍCTOR VIRGÍLIO DA SILVA	2920
DA SILVA, K.C.D.	KAREN CRISTINA DALLAQUA DA SILVA	3358
DA SILVA, L.C.	LUAN CRISTIAN DA SILVA	2847
DA SILVA, L.E.M.	LAURA EDUARDA MIRANDA DA SILVA	2695
DA SILVA, L.E.M.	LAURA EDUARDA MIRANDA DA SILVA	2700
DA SILVA, L.F.F.	LUIZ FERNANDO FERRAZ DA SILVA	2792
DA SILVA, L.F.F.	LUIZ FERNANDO FERRAZ DA SILVA	3020
DA SILVA, L.G.	LUCAS GOMES DA SILVA	2756
DA SILVA, L.G.	LUCAS GOMES DA SILVA	3267
DA SILVA, L.H.P.	LUCIA HELENA PINTO DA SILVA	2912
DA SILVA, L.H.P.	LUCIA HELENA PINTO DA SILVA	2918
DA SILVA, L.H.P.	LUCIA HELENA PINTO DA SILVA	2936
DA SILVA, L.H.P.	LUCIA HELENA PINTO DA SILVA	2948
DA SILVA, L.H.R.	LUCAS HENRIQUE RODRIGUES DA SILVA	3226
DA SILVA, L.H.R.	LUCAS HENRIQUE RODRIGUES DA SILVA	3236
DA SILVA, L.I.	LARISSA IGNÁCIO DA SILVA	2952
DA SILVA, L.L.F.	LETÍCIA LOPES FRANCA DA SILVA	2757
DA SILVA, L.O.	LARISSA OLIVEIRA DA SILVA	2779
DA SILVA, L.O.	LARISSA OLIVEIRA DA SILVA	3210
DA SILVA, L.P.	LUCIANA PINHEIRO DA SILVA	2936
DA SILVA, L.P.	LUCIANA PINHEIRO DA SILVA	2948
DA SILVA, L.R.	LARA RODRIGUES DA SILVA	3020
DA SILVA, L.T.	LAIS TEODORO DA SILVA	2776
DA SILVA, L.T.P.	LUCAS TINÉ PEREIRA DA SILVA	2930
DA SILVA, M.A.	MINELLY AZEVEDO DA SILVA	3514
DA SILVA, M.B.	MÁRCIA BARBOSA DA SILVA	3373
DA SILVA, M.B.	MÁRCIA BARBOSA DA SILVA	3406

DA SILVA, M.B.	MÁRCIA BARBOSA DA SILVA	3441
DA SILVA, M.B.	MÁRCIA BARBOSA DA SILVA	3504
DA SILVA, M.C.	MARIA CLÁUDIA DA SILVA	3376
DA SILVA, M.C.F.	MÁRCIA CRISTINA FREITAS DA SILVA	3408
DA SILVA, M.D.S.T.	MILENA DOS SANTOS TAVARES DA SILVA	2856
DA SILVA, M.D.S.T.	MILENA DOS SANTOS TAVARES DA SILVA	2907
DA SILVA, M.H.P.	MATHEUS HENRIQUE PEREIRA DA SILVA	3468
DA SILVA, M.L.M.	MARIA LUIZA MENEZES DA SILVA	3386
DA SILVA, M.S.P.	MILLA SOUZA PESSOA DA SILVA	3467
DA SILVA, M.S.P.	MILLA SOUZA PESSOA DA SILVA	3491
DA SILVA, M.S.P.	MILLA SOUZA PESSOA DA SILVA	3494
DA SILVA, M.T.L.	MAYARA TORQUATO LIMA DA SILVA	3095
DA SILVA, M.T.T.	MARCUS TULIUS TEIXEIRA DA SILVA	3145
DA SILVA, M.T.T.	MARCUS TULIUS TEIXEIRA DA SILVA	3425
DA SILVA, N.J.	NATÁLIA JEANEGITZ DA SILVA	2916
DA SILVA, P.	PATRICK DA SILVA	2889
DA SILVA, P.B.	PATRICIA BRAGA DA SILVA	2985
DA SILVA, P.B.	PATRICIA BRAGA DA SILVA	3222
DA SILVA, P.S.	PAULA SIMPLÍCIO DA SILVA	3404
DA SILVA, R.N.R.	RODRIGO NUNES RODRIGUES DA SILVA	3050
DA SILVA, R.N.R.	RODRIGO NUNES RODRIGUES DA SILVA	3355
DA SILVA, R.P.	RAFAELA PIRES DA SILVA	3321
DA SILVA, R.P.	RAYZZA PESSANHA DA SILVA	2728
DA SILVA, R.T.B.	RAQUEL TAVARES BOY DA SILVA	2742
DA SILVA, T.A.	THIAGO APARECIDO DA SILVA	3173
DA SILVA, T.A.	THIAGO APARECIDO DA SILVA	3202
DA SILVA, T.D.C.M.	THAYANE DA CONCEIÇÃO MATOS DA SILVA	2892
DA SILVA, V.A.C.	VINICIUS ALEXANDRE CORDEIRO DA SILVA	3164
DA SILVA, V.J.	VIVIANE JENNIFER DA SILVA	2854
DA SILVA, V.J.	VIVIANE JENNIFER DA SILVA	2855
DA SILVA, V.J.	VIVIANE JENNIFER DA SILVA	3221
DA SILVA, W.N.	WALISON NUNES DA SILVA	3032
DA SILVA-JUNIOR, E.B.	ELIAS BARBOSA DA SILVA-JUNIOR	3232
DA SILVA-JUNIOR, E.B.	ELIAS BARBOSA DA SILVA-JUNIOR	3418
DA SILVEIRA, A.G.	ANTHONY GABRY DA SILVEIRA	2917
DA VEIGA, G.T.S.	GISELE TATIANE SOARES DA VEIGA	2836
DACRI, I.M.	ISABELLA MARINS DACRI	2818
DACRI, I.M.	ISABELLA MARINS DACRI	2963
DACRI, I.M.	ISABELLA MARINS DACRI	3471
DA-CRUZ, A.M.	ALDA MARIA DA-CRUZ	2823
DAHER, I.P.	ISABELA PAZOTTI DAHER	2740
DAHER, I.P.	ISABELA PAZOTTI DAHER	3149
DALEY, G.Q.	GEORGE QUENTIN DALEY	2762
DALLAZUANA, V.	VITÓRIA DALLAZUANA	2857
DALTRO, J.D.P.	JULIANA DA PAIXÃO DALTRO	2871
DALTRO, J.D.P.	JULIANA DA PAIXÃO DALTRO	2887
DALTRO, J.D.P.	JULIANA DA PAIXÃO DALTRO	2910
DALTRO, J.D.P.	JULIANA DA PAIXÃO DALTRO	3025
DALTRO, S.R.T.	SERGIO RICARDO TEIXEIRA DALTRO	3370
DAMAS, M.S.F.	MARCELO SILVA FOLHAS DAMAS	2867
DAMAS, M.S.F.	MARCELO SILVA FOLHAS DAMAS	3018
DAMASCENO, L.E.A.	LUIS EDUARDO ALVES DAMASCENO	2750
DAMASCENO, L.E.A.	LUIS EDUARDO ALVES DAMASCENO	2755
DAMASCENO, L.E.A.	LUIS EDUARDO ALVES DAMASCENO	3088
DAMASCENO, L.E.A.	LUIS EDUARDO ALVES DAMASCENO	3097
DAMASCENO, L.E.A.	LUIS EDUARDO ALVES DAMASCENO	3170
DAMASCENO, L.E.A.	LUIS EDUARDO ALVES DAMASCENO	3333
DAMASCENO, L.E.A.	LUIS EDUARDO ALVES DAMASCENO	3415
DAMASCENO, S.	SAMARA DAMASCENO	3369

DANIELLI, C.R.	CAMILA RAMOS DANIELLI	3368
DANIEL-RIBEIRO, C.T.	CLÁUDIO TADEU DANIEL-RIBEIRO	2743
DANIEL-RIBEIRO, C.T.	CLÁUDIO TADEU DANIEL-RIBEIRO	2786
DANIEL-RIBEIRO, C.T.	CLÁUDIO TADEU DANIEL-RIBEIRO	2849
DANIEL-RIBEIRO, C.T.	CLÁUDIO TADEU DANIEL-RIBEIRO	2893
DANIEL-RIBEIRO, C.T.	CLÁUDIO TADEU DANIEL-RIBEIRO	2940
DANIEL-RIBEIRO, C.T.	CLÁUDIO TADEU DANIEL-RIBEIRO	3355
DANIEL-RIBEIRO, C.T.	CLÁUDIO TADEU DANIEL-RIBEIRO	3356
DANTAS, B.A.	BIANCA ALVES DANTAS	3264
DANTAS, D.P.	DANILLO PEREIRA DANTAS	3502
DANTAS, F.B.	FABRÍCIO BRITO DANTAS	3282
DANTAS, V.A.N.C.	VIVIANE ABREU NUNES CERQUEIRA DANTAS	2835
DARCY, P.	PATRICK DARCY	2798
DARDENNE, L.E.	LAURENT EMMANUEL DARDENNE	3251
DAVANZO, G.G.	GUSTAVO GASTÃO DAVANZO	2772
DAVANZO, G.G.	GUSTAVO GASTÃO DAVANZO	2775
DAVANZO, G.G.	GUSTAVO GASTÃO DAVANZO	2882
DAVID, B.A.	BRUNA ARAUJO DAVID	2853
DAVID, R.B.	RENATA BERNARDES DAVID	3291
DAVIES, J.	JESSICA DAVIES	2844
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2734
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2735
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2741
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2801
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2878
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2889
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2938
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2966
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2967
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	2982
DE ABREU, A.M.	ADRIAN MARTOS DE ABREU	3076
DE AGUIAR, A.M.	ANANDA MAGALHÃES DE AGUIAR	3173
DE ALBUQUERQUE, C.F.G.	CASSIANO FELIPPE GONÇALVES DE ALBUQUERQUE	3467
DE ALENCAR, B.C.	BRUNA CUNHA DE ALENCAR	3089
DE ALENCAR, B.C.	BRUNA CUNHA DE ALENCAR	3091
DE ALENCAR, R.E.	RAQUEL ELAINE DE ALENCAR	3149
DE ALENCAR, S.M.	SEVERINO MATIAS DE ALENCAR	3003
DE ALMEIDA, C.D.	CAMILA DIAS DE ALMEIDA	3184
DE ALMEIDA, C.D.	CAMILA DIAS DE ALMEIDA	3249
DE ALMEIDA, C.D.	CAMILA DIAS DE ALMEIDA	3411
DE ALMEIDA, D.B.	DOUGLAS BARROSO DE ALMEIDA	3343
DE ALMEIDA, D.B.	DOUGLAS BARROSO DE ALMEIDA	3349
DE ALMEIDA, D.B.	DOUGLAS BARROSO DE ALMEIDA	3507
DE ALMEIDA, D.C.	DANILO CANDIDO DE ALMEIDA	2903
DE ALMEIDA, D.V.	DALZIZA VICTALINA DE ALMEIDA	3000
DE ALMEIDA, D.V.	DALZIZA VICTALINA DE ALMEIDA	3105
DE ALMEIDA, G.J.M.	GUSTAVO JOSÉ MAKHOUL DE ALMEIDA	2738
DE ALMEIDA, G.J.M.	GUSTAVO JOSÉ MAKHOUL DE ALMEIDA	3232
DE ALMEIDA, G.J.M.	GUSTAVO JOSÉ MAKHOUL DE ALMEIDA	3418
DE ALMEIDA, J.A.	JÚLIA ANDRESSA DE ALMEIDA	2749
DE ALMEIDA, J.A.	JÚLIA ANDRESSA DE ALMEIDA	2934
DE ALMEIDA, J.R.	JOSÉ RAFAEL DE ALMEIDA	3503
DE ALMEIDA, L.	LETÍCIA DE ALMEIDA	3465
DE ALMEIDA, L.A.	LEONARDO AUGUSTO DE ALMEIDA	2962
DE ALMEIDA, L.A.	LEONARDO AUGUSTO DE ALMEIDA	2965
DE ALMEIDA, L.A.	LEONARDO AUGUSTO DE ALMEIDA	3003
DE ALMEIDA, M.F.	MATHEUS FERREIRA DE ALMEIDA	2761
DE ALMEIDA, M.F.	MATHEUS FERREIRA DE ALMEIDA	2771
DE ALMEIDA, M.F.	MATHEUS FERREIRA DE ALMEIDA	2791

DE ALMEIDA, M.F.	MATHEUS FERREIRA DE ALMEIDA	2793
DE ALMEIDA, P.E.	PATRICIA ELAINE DE ALMEIDA	3352
DE ALMEIDA, T.C.	THAÍS COSTA DE ALMEIDA	3147
DE ALMEIDA, T.F.	TATIANA FERREIRA DE ALMEIDA	3060
DE ALMEIDA, V.M.	VIVIANI MENDES DE ALMEIDA	2699
DE ALMEIDA, V.M.	VIVIANI MENDES DE ALMEIDA	2970
DE ALMEIDA, V.M.	VIVIANI MENDES DE ALMEIDA	3337
DE ALVARENGA, V.G.	VALÉRIA GONÇALVES DE ALVARENGA	2981
DE ALVARENGA, V.G.	VALÉRIA GONÇALVES DE ALVARENGA	2993
DE AMORIM, C.S.	CAROLINNE SOUZA DE AMORIM	2925
DE AMORIM, L.H.	LEANDRO HONORATO DE AMORIM	2815
DE AMORIM, L.H.	LEANDRO HONORATO DE AMORIM	3090
DE AMORIM, L.H.	LEANDRO HONORATO DE AMORIM	3484
DE AMORIM, N.S.	NYCOLE SOUZA DE AMORIM	2925
DE ANDRADE, A.C.M.M.	ANA CLARA MATOSO MONTUORI DE ANDRADE	2914
DE ANDRADE, A.C.M.M.	ANA CLARA MATOSO MONTUORI DE ANDRADE	2997
DE ANDRADE, A.C.M.M.	ANA CLARA MATOSO MONTUORI DE ANDRADE	3513
DE ANDRADE, B.W.	BRUNO WANGHAN DE ANDRADE	3323
DE ANDRADE, C.M.	CANDACE MACHADO DE ANDRADE	3312
DE ANDRADE, C.V.	CECÍLIA VIANNA DE ANDRADE	2901
DE ANDRADE, C.V.	CECÍLIA VIANNA DE ANDRADE	3058
DE ANDRADE, E.A.M.	EMANOELA ALINE MORAIS DE ANDRADE	3005
DE ANDRADE, G.P.	GLAUCIA PRADO DE ANDRADE	3117
DE ANDRADE, G.P.	GLAUCIA PRADO DE ANDRADE	3368
DE ANDRADE, J.C.D.C.	JESSICA CRISTIANE DA CONCEIÇÃO DE ANDRADE	2850
DE ANDRADE, J.C.D.C.	JESSICA CRISTIANE DA CONCEIÇÃO DE ANDRADE	3182
DE ANDRADE, J.C.D.C.	JESSICA CRISTIANE DA CONCEIÇÃO DE ANDRADE	3197
DE ANDRADE, J.C.D.C.	JESSICA CRISTIANE DA CONCEIÇÃO DE ANDRADE	3366
DE ANDRADE, L.P.	LARISSA PINTO DE ANDRADE	2852
DE ANDRADE, L.P.	LARISSA PINTO DE ANDRADE	3022
DE ARAUJO, A.C.V.S.C.	ANA CAROLINA VALENTE SANTOS CRUZ DE ARAUJO	2781
DE ARAUJO, E.G.	ELIZABETH GIESTAL DE ARAUJO	2758
DE ARAUJO, F.M.D.S.	FABIELLE MARQUES DOS SANTOS DE ARAUJO	3359
DE ARAUJO, F.M.D.S.	FABIELLE MARQUES DOS SANTOS DE ARAUJO	3404
DE ARAÚJO, L.E.	LUANA EVANGELISTA DE ARAÚJO	2946
DE ARAÚJO, L.P.	LEONARDO PEREIRA DE ARAÚJO	2962
DE ARAÚJO, L.P.	LEONARDO PEREIRA DE ARAÚJO	2965
DE ARAÚJO, L.P.	LEONARDO PEREIRA DE ARAÚJO	3003
DE ARAÚJO, M.C.	MATHEUS CARNEIRO DE ARAÚJO	3119
DE ARAÚJO, M.N.V.	MILENA NASCIMENTO VERDAM DE ARAÚJO	3069
DE ARAÚJO, M.P.	MATHEUS PEREIRA DE ARAÚJO	3246
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2734
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2735
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2741
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2878
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2889
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2938
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2944
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2966
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2967
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	2982
DE ARAUJO, M.V.	MARCELO VALDEMIR DE ARAUJO	3076
DE ARAÚJO, P.H.H.	PEDRO HENRIQUE HERMES DE ARAÚJO	3499
DE ARAUJO, T.L.	TALITA LACERDA DE ARAUJO	3399
DE ARAUJO, V.F.	VANESSA FERREIRA DE ARAUJO	2983
DE ARAUJO, W.G.	WILLIAN GOMES DE ARAUJO	3014
DE ARAUJO, W.G.	WILLIAN GOMES DE ARAUJO	3072
DE ARAÚJO-JORGE, T.C.	TANIA C DE ARAÚJO-JORGE	3359
DE ARAÚJO-JORGE, T.C.	TANIA C DE ARAÚJO-JORGE	3404

DE ARRUDA, L.B.	LUCIANA BARROS DE ARRUDA	2898
DE ARRUDA, L.B.	LUCIANA BARROS DE ARRUDA	3494
DE ASSIS, H.C.	HELDER CARVALHO DE ASSIS	2673
DE ASSIS, H.C.	HELDER CARVALHO DE ASSIS	2798
DE ASSIS, H.C.	HELDER CARVALHO DE ASSIS	2869
DE ASSIS, H.C.	HELDER CARVALHO DE ASSIS	3220
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2734
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2741
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2878
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2889
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2938
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2944
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2966
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2967
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	2982
DE ASSIS, J.B.	JOSIANE BETIM DE ASSIS	3076
DE ASSIS, L.M.	LUAN MATHEUS DE ASSIS	2786
DE ASSIS, L.M.	LUAN MATHEUS DE ASSIS	2849
DE ASSUMPTÃO, P.P.	PAULO PIMENTEL DE ASSUMPTÃO	3048
DE ASSUNÇÃO, S.F.V.	STELLA FRANCY VICENTE DE ASSUNÇÃO	3372
DE AZEVEDO, A.C.	AMILCAR CARDOSO DE AZEVEDO	2751
DE AZEVEDO, P.O.	PATRICK ORESTES DE AZEVEDO	2658
DE AZEVEDO, S.S.D.	SUWELLEN S. D. DE AZEVEDO	2872
DE AZEVEDO, S.S.D.	SUWELLEN S. D. DE AZEVEDO	3064
DE AZEVEDO, S.S.D.	SUWELLEN S. D. DE AZEVEDO	3409
DE AZEVEDO, V.M.	VITÓRIA MONTEIRO DE AZEVEDO	2786
DE AZEVEDO, V.M.	VITÓRIA MONTEIRO DE AZEVEDO	2849
DE AZEVEDO, Y.G.	YAGO GONÇALVES DE AZEVEDO	2970
DE BARROS, H.L.P.	HELENA LOMBARDI PARENTE DE BARROS	2665
DE BARROS, H.L.P.	HELENA LOMBARDI PARENTE DE BARROS	2677
DE BARROS, H.L.P.	HELENA LOMBARDI PARENTE DE BARROS	2682
DE BARROS, J.P.	JENIFER PEIXOTO DE BARROS	2893
DE BARROS, L.A.K.	LARISSA AKEMI KIDO DE BARROS	2947
DE BARROS, R.R.	RAFAEL RODRIGUES DE BARROS	2663
DE BEM, A.F.	ANDREZA FABRO DE BEM	3001
DE BRITO, J.P.	JOÃO PEDRO DE BRITO	3245
DE BROT, N.C.	NATHALIA COIMBRA DE BROT	2650
DE CAMARGO, I.M.	ISABELLA MITIE DE CAMARGO	3225
DE CAMPOS, C.L.V.	CAROLINE LEONEL VASCONCELOS DE CAMPOS	2640
DE CAMPOS, E.M.	EMANUELLE MARTINS DE CAMPOS	2782
DE CAMPOS, I.B.	IVANA BARROS DE CAMPOS	3002
DE CAMPOS, M.M.	MARIANA MACEDO DE CAMPOS	3426
DE CAMPOS, M.M.	MARIANA MACEDO DE CAMPOS	3490
DE CAMPOS, N.S.P.	NAJLA SANTOS PACHECO DE CAMPOS	3039
DE CAMPOS, N.S.P.	NAJLA SANTOS PACHECO DE CAMPOS	3290
DE CARVALHO FILHO, E.M.	EDGAR MARCELINO DE CARVALHO FILHO	2828
DE CARVALHO FILHO, E.M.	EDGAR MARCELINO DE CARVALHO FILHO	3075
DE CARVALHO FILHO, E.M.	EDGAR MARCELINO DE CARVALHO FILHO	3125
DE CARVALHO FILHO, E.M.	EDGAR MARCELINO DE CARVALHO FILHO	3210
DE CARVALHO FILHO, E.M.	EDGAR MARCELINO DE CARVALHO FILHO	3231
DE CARVALHO FILHO, E.M.	EDGAR MARCELINO DE CARVALHO FILHO	3432
DE CARVALHO FILHO, E.M.	EDGAR MARCELINO DE CARVALHO FILHO	3489
DE CARVALHO, A.S.	ANA SALLES DE CARVALHO	3304
DE CARVALHO, A.T.	ANDRÉA TEIXEIRA DE CARVALHO	2642
DE CARVALHO, A.T.	ANDRÉA TEIXEIRA DE CARVALHO	2650
DE CARVALHO, A.T.	ANDRÉA TEIXEIRA DE CARVALHO	2677
DE CARVALHO, A.T.	ANDRÉA TEIXEIRA DE CARVALHO	2715
DE CARVALHO, A.T.	ANDRÉA TEIXEIRA DE CARVALHO	2976
DE CARVALHO, B.O.M.	BEATRIZ OLIVEIRA MOREIRA DE CARVALHO	2852

DE CARVALHO, C.	CAROLINE DE CARVALHO	2778
DE CARVALHO, C.	CAROLINE DE CARVALHO	3411
DE CARVALHO, D.M.	DIEGO MONTEIRO DE CARVALHO	3445
DE CARVALHO, F.A.S.	FLÁVIO AIMBIRE SOARES DE CARVALHO	2939
DE CARVALHO, G.B.F.	GARDENIA BRAZ FIGUEIREDO DE CARVALHO	2646
DE CARVALHO, G.M.S.	GIOVANA MARIA SALMAZI DE CARVALHO	2695
DE CARVALHO, G.M.S.	GIOVANA MARIA SALMAZI DE CARVALHO	2696
DE CARVALHO, G.M.S.	GIOVANA MARIA SALMAZI DE CARVALHO	2700
DE CARVALHO, J.C.S.	JONATAN CONSTANÇA SILVA DE CARVALHO	2861
DE CARVALHO, J.C.S.	JONATAN CONSTANÇA SILVA DE CARVALHO	2959
DE CARVALHO, J.C.S.	JONATAN CONSTANÇA SILVA DE CARVALHO	2969
DE CARVALHO, J.V.	JULIANNE VARGAS DE CARVALHO	3016
DE CARVALHO, L.D.	LUCIANA DEBORTOLI DE CARVALHO	2693
DE CARVALHO, L.I.A.	LUANA IZABELA AZEVEDO DE CARVALHO	3445
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	2828
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	2862
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	2868
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	2921
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3075
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3098
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3125
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3167
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3192
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3209
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3210
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3231
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3372
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3489
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3517
DE CARVALHO, L.P.	LUCAS PEDREIRA DE CARVALHO	3520
DE CARVALHO, M.E.F.	MARIA EDUARDA FERRAZ DE CARVALHO	3522
DE CARVALHO, M.F.	MIRIAN FELIX DE CARVALHO	3344
DE CARVALHO, M.M.	MILENA MORAES DE CARVALHO	3013
DE CARVALHO, M.M.	MILENA MORAES DE CARVALHO	3299
DE CARVALHO, M.M.	MILENA MORAES DE CARVALHO	3301
DE CARVALHO, M.R.M.	MARCIO ROBERTO MORAES DE CARVALHO	2998
DE CARVALHO, M.V.	MÁRCIA VIDAL DE CARVALHO	3329
DE CARVALHO, P.C.S.	POLIANA CAPUCHO SANDRE DE CARVALHO	2931
DE CARVALHO, Y.B.R.	YURI BIGIO RIBEIRO DE CARVALHO	3335
DE CASTRO, C.	CAMILLA DE CASTRO	3000
DE CASTRO, C.H.D.F.	CARLOS HENRIQUE DETTMANN FANTECELLE DE CASTRO	2862
DE CASTRO, C.H.D.F.	CARLOS HENRIQUE DETTMANN FANTECELLE DE CASTRO	2868
DE CASTRO, C.H.D.F.	CARLOS HENRIQUE DETTMANN FANTECELLE DE CASTRO	3059
DE CASTRO, F.A.	FABIOLA ATTIE DE CASTRO	3285
DE CASTRO, G.	GISELE DE CASTRO	2831
DE CASTRO, G.	GISELE DE CASTRO	2882
DE CASTRO, J.T.	JULIA TEIXEIRA DE CASTRO	2641
DE CASTRO, J.T.	JULIA TEIXEIRA DE CASTRO	2651
DE CASTRO, K.K.G.	KATHERINE KELDA GOMES DE CASTRO	3356
DE CASTRO, L.F.	LIVIA FURQUIM DE CASTRO	3221
DE CASTRO, L.F.	LIVIA FURQUIM DE CASTRO	3236
DE CASTRO, M.M.	MARINA MOREIRA DE CASTRO	3067
DE CASTRO, N.S.	NATÁLIA SALAZAR DE CASTRO	2641
DE CASTRO, R.J.A.	RAFFAEL JÚNIO ARAÚJO DE CASTRO	3079
DE CASTRO, S.B.R.	SANDRA BERTELLI RIBEIRO DE CASTRO	3046
DE CASTRO, S.B.R.	SANDRA BERTELLI RIBEIRO DE CASTRO	3051
DE CASTRO, Y.S.	YURI SHEIDEGGER DE CASTRO	3377
DE CORREIA, C.R.S.T.B.	CAMILA ROLEMBERG SANTANA TRAVAGLINI BERTI DE CORREIA	3255
DE FARIAS, I.S.	INGRID SANCHO DE FARIAS	3423

DE FARIAS, K.C.R.M.	KELEN CRISTINA RIBEIRO MALMEGRIM DE FARIAS	3120
DE FELICE, F.G.	FERNANDA G. DE FELICE	2816
DE FIGUEIREDO, A.B.	AMANDA BRAGA DE FIGUEIREDO	3133
DE FIGUEIREDO, C.P.	CLAUDIA PINTO DE FIGUEIREDO	3491
DE FIGUEIREDO, C.P.	CLAUDIA PINTO DE FIGUEIREDO	3494
DE FIGUEIREDO, D.	DANIELLA DE FIGUEIREDO	2733
DE FRANÇA, F.S.	FELIPE SILVA DE FRANÇA	3319
DE FRANCO, M.T.	MILENE TINO DE FRANCO	2894
DE FREITAS, D.	DENISE DE FREITAS	2759
DE FREITAS, D.D.N.	DEISE DO NASCIMENTO DE FREITAS	3031
DE FREITAS, G.M.	GIOVANA MAGALHÃES DE FREITAS	2698
DE FREITAS, G.M.	GIOVANA MAGALHÃES DE FREITAS	2699
DE FREITAS, G.M.	GIOVANA MAGALHÃES DE FREITAS	2702
DE FREITAS, K.H.	KIANNI HERNANDEZ DE FREITAS	3077
DE FREITAS, L.R.C.	LAYLA RICARDO CIDINHO DE FREITAS	2941
DE FREITAS, L.R.C.	LAYLA RICARDO CIDINHO DE FREITAS	2977
DE GASPARI, E.	ELIZABETH DE GASPARI	2906
DE GASPARI, E.	ELIZABETH DE GASPARI	3002
DE GASPARI, E.	ELIZABETH DE GASPARI	3011
DE GASPARI, E.	ELIZABETH DE GASPARI	3042
DE GASPARI, E.	ELIZABETH DE GASPARI	3061
DE GASPARI, E.	ELIZABETH DE GASPARI	3096
DE GASPARI, E.N.	ELIZABETH NATAL DE GASPARI	3450
DE GASPERI, R.M.	REBECCA MELLO DE GASPERI	2737
DE GASPERI, R.M.	REBECCA MELLO DE GASPERI	3092
DE GODOY, D.T.	DANIELA TUPY DE GODOY	3142
DE GODOY, K.F.	KRISSIA FRANCO DE GODOY	3297
DE GOUVEIA, V.L.C.	VERA LUCIA CONCEIÇÃO DE GOUVEIA	2954
DE HOLANDA, K.E.R.	KEYLA EMANUELLE RAMOS DE HOLANDA	3514
DE HOLANDA, M.T.	MARCELO TEIXEIRA DE HOLANDA	3359
DE HOLANDA, M.T.	MARCELO TEIXEIRA DE HOLANDA	3404
DE JESUS, A.C.V.	ANA CAROLINA VIEIRA DE JESUS	3052
DE JESUS, A.C.V.	ANA CAROLINA VIEIRA DE JESUS	3057
DE JESUS, M.D.S.	MILENA DA SILVA DE JESUS	3344
DE JESUS, T.A.	THIAGO ALVES DE JESUS	3303
DE JESUS, T.A.	THIAGO ALVES DE JESUS	3307
DE JESUS, T.S.	THAYSE SILVA DE JESUS	2871
DE JESUS, T.S.	THAYSE SILVA DE JESUS	2887
DE JESUS, T.S.	THAYSE SILVA DE JESUS	2910
DE JESUS, T.S.	THAYSE SILVA DE JESUS	3025
DE JESUS, T.S.	THAYSE SILVA DE JESUS	3520
DE LACERDA, L.B.	LUNA BARROCO DE LACERDA	2632
DE LACERDA, L.B.	LUNA BARROCO DE LACERDA	2633
DE LACERDA, L.B.	LUNA BARROCO DE LACERDA	2648
DE LACERDA, L.B.	LUNA BARROCO DE LACERDA	3347
DE LACERDA, L.B.	LUNA BARROCO DE LACERDA	3509
DE LANA, M.	MARTA DE LANA	2630
DE LEMOS, E.R.S.	ELBA REGINA SAMPAIO DE LEMOS	3431
DE LIMA, A.B.	AMANDA BARROS DE LIMA	3323
DE LIMA, A.B.F.	ANA BEATRIZ FIGUEIREDO DE LIMA	3229
DE LIMA, A.C.S.	ANA CAROLINA SOUZA DE LIMA	3064
DE LIMA, A.M.	ANDERSON MACIEL DE LIMA	2971
DE LIMA, B.C.	BEATRIZ CARVALHO DE LIMA	3014
DE LIMA, B.C.	BEATRIZ CARVALHO DE LIMA	3072
DE LIMA, B.R.S.	BEATRIZ RIBEIRO SALVADOR DE LIMA	3399
DE LIMA, E.H.R.	ERIC HENRIQUE ROMA DE LIMA	3058
DE LIMA, E.H.R.	ERIC HENRIQUE ROMA DE LIMA	3404
DE LIMA, E.H.R.	ERIC HENRIQUE ROMA DE LIMA	3505
DE LIMA, F.M.D.S.	FERNANDO MESQUITA DE SOUSA DE LIMA	2751

DE LIMA, F.M.D.S.	FERNANDO MESQUITA DE SOUSA DE LIMA	2947
DE LIMA, F.M.D.S.	FERNANDO MESQUITA DE SOUSA DE LIMA	2949
DE LIMA, F.M.D.S.	FERNANDO MESQUITA DE SOUSA DE LIMA	2973
DE LIMA, J.D.	JORDANA DINORÁ DE LIMA	2836
DE LIMA, J.E.N.	JULIANA ECHEVARRIA NEVES DE LIMA	2941
DE LIMA, J.E.N.	JULIANA ECHEVARRIA NEVES DE LIMA	2963
DE LIMA, J.E.N.	JULIANA ECHEVARRIA NEVES DE LIMA	2977
DE LIMA, J.E.N.	JULIANA ECHEVARRIA NEVES DE LIMA	3145
DE LIMA, J.E.N.	JULIANA ECHEVARRIA NEVES DE LIMA	3425
DE LIMA, J.O.	JULIA OLIVEIRA DE LIMA	2867
DE LIMA, J.O.	JULIA OLIVEIRA DE LIMA	3018
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	2897
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	2904
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	3099
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	3132
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	3138
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	3195
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	3238
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	3241
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	3256
DE LIMA, J.V.C.S.	JOÃO VICTOR CONCEIÇÃO SOARES DE LIMA	3261
DE LIMA, L.	LUANA DE LIMA	3135
DE LIMA, L.C.	LOUISE CORREIA DE LIMA	3312
DE LIMA, L.D.C.	LAÍS DOMINGOS COSTA DE LIMA	2888
DE LIMA, L.F.	LEONARDO FREIRE DE LIMA	2738
DE LIMA, L.F.	LEONARDO FREIRE DE LIMA	3484
DE LIMA, L.G.R.	LUIZ GUSTAVO RIBEIRO DE LIMA	3360
DE LIMA, L.N.	LUANA NETO DE LIMA	3108
DE LIMA, M.F.C.	MARIO FLÁVIO CARDOSO DE LIMA	3280
DE LIMA, M.F.C.	MARIO FLÁVIO CARDOSO DE LIMA	3302
DE LIMA, N.C.R.	NATHAN COGLIATTI RIBEIRO DE LIMA	3290
DE LIMA, S.C.G.	SARAH CAROLINE GOMES DE LIMA	2870
DE LIMA, S.C.G.	SARAH CAROLINE GOMES DE LIMA	2959
DE LIMA, S.C.G.	SARAH CAROLINE GOMES DE LIMA	2969
DE LIMA, S.C.G.	SARAH CAROLINE GOMES DE LIMA	3016
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	2827
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	2841
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	2842
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	3029
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	3094
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	3121
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	3134
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	3171
DE LIMA, V.C.C.	VLADMIR CLÁUDIO CORDEIRO DE LIMA	3309
DE LIMA, W.T.	WOTHAN TAVARES DE LIMA	2894
DE LIRA, G.S.	GUILHERME SANT'ANNA DE LIRA	2719
DE LIRA, G.S.	GUILHERME SANT'ANNA DE LIRA	2818
DE LIRA, G.S.	GUILHERME SANT'ANNA DE LIRA	2963
DE LISBOA, M.F.S.	MARIA FERNANDA SOARES DE LISBOA	2649
DE LUCA, D.M.	DANIELA MACHADO DE LUCA	3240
DE LUCA, D.M.	DANIELA MACHADO DE LUCA	3260
DE LUCA, P.M.	PAULA MELLO DE LUCA	3050
DE LUCA, P.M.	PAULA MELLO DE LUCA	3356
DE LUCENA, V.W.L.	VICTORIA WEISE LEITE DE LUCENA	3212
DE MACEDO, C.S.	CRISTIANA SANTOS DE MACEDO	3228
DE MAGALHÃES, P.C.	PAULA CRISTINA DE MAGALHÃES	3454
DE MATOS, A.L.T.	ANA LUIZA TANCREDI DE MATOS	3053
DE MATOS, A.L.T.	ANA LUIZA TANCREDI DE MATOS	3389
DE MATOS, C.M.	CAROLINE MARTINS DE MATOS	3416

DE MATOS, J.A.	JULIANA ARRUDA DE MATOS	3105
DE MATOS, J.P.S.	JORGE PAULO STROGOFF DE MATOS	3006
DE MATOS, L.V.	LORENA VIEIRA DE MATOS	2727
DE MATTOS, G.F.P.	GABRIEL FRANCISCO POZZO DE MATTOS	3353
DE MATTOS, G.F.P.	GABRIEL FRANCISCO POZZO DE MATTOS	3488
DE MATTOS-GUARALDI, A.L.	ANA LUIZA DE MATTOS-GUARALDI	2874
DE MATTOS-GUARALDI, A.L.	ANA LUIZA DE MATTOS-GUARALDI	2911
DE MEDEIROS, A.I.	ALEXANDRA IVO DE MEDEIROS	3189
DE MEDEIROS, A.I.	ALEXANDRA IVO DE MEDEIROS	3258
DE MEDEIROS, A.I.	ALEXANDRA IVO DE MEDEIROS	3283
DE MEDEIROS, A.I.	ALEXANDRA IVO DE MEDEIROS	3459
DE MEDEIROS, A.I.	ALEXANDRA IVO DE MEDEIROS	3460
DE MEDEIROS ³ , V.P.	VALQUÍRIA PEREIRA DE MEDEIROS ³ .	3354
DE MEIRA, T.A.	TIFANY ALVES DE MEIRA	2698
DE MEIRA, T.A.	TIFANY ALVES DE MEIRA	2702
DE MELLO, I.M.A.	ISIS MAIA APOLINARIO DE MELLO	2961
DE MELLO, I.M.A.	ISIS MAIA APOLINARIO DE MELLO	3449
DE MELO, B.M.S.	BRUNO MARCEL SILVA DE MELO	2755
DE MELO, B.M.S.	BRUNO MARCEL SILVA DE MELO	3088
DE MELO, B.M.S.	BRUNO MARCEL SILVA DE MELO	3097
DE MELO, B.M.S.	BRUNO MARCEL SILVA DE MELO	3170
DE MELO, B.M.S.	BRUNO MARCEL SILVA DE MELO	3333
DE MELO, B.M.S.	BRUNO MARCEL SILVA DE MELO	3415
DE MELO, C.B.	CHRISTIANNE BANDEIRA DE MELO	2885
DE MELO, C.B.	CHRISTIANNE BANDEIRA DE MELO	2919
DE MELO, C.B.	CHRISTIANNE BANDEIRA DE MELO	3014
DE MELO, C.B.	CHRISTIANNE BANDEIRA DE MELO	3274
DE MELO, C.B.	CHRISTIANNE BANDEIRA DE MELO	3328
DE MELO, D.K.C.	DAMILA KAREN CARDOSO DE MELO	3452
DE MELO, D.K.C.	DAMILA KAREN CARDOSO DE MELO	3511
DE MELO, H.A.B.	HELOÍSA ANTONIELLA BRAZ DE MELO	3001
DE MELO, H.A.B.	HELOÍSA ANTONIELLA BRAZ DE MELO	3313
DE MELO, M.A.F.	MARÍLIA ALVES FIGUEIRA DE MELO	3065
DE MELO, M.A.F.	MARÍLIA ALVES FIGUEIRA DE MELO	3371
DE MELO, N.S.	NICOLY SIMÕES DE MELO	3011
DE MELO, N.S.	NICOLY SIMÕES DE MELO	3061
DE MELO, P.	PAULO DE MELO	3269
DE MELO, P.	PAULO DE MELO	3320
DE MELO, T.F.	THIFANY FOSCHIERA DE MELO	3514
DE MELO, Y.L.M.F.	YARA LUCIA MENDES FURTADO DE MELO	3058
DE MENDONÇA, V.R.R.	VITOR ROSA RAMOS DE MENDONÇA	2991
DE MENEZES, J.P.B.	JULIANA PERRONE BEZERRA DE MENEZES	2775
DE MENEZES, J.P.B.	JULIANA PERRONE BEZERRA DE MENEZES	3156
DE MENEZES, J.P.B.	JULIANA PERRONE BEZERRA DE MENEZES	3291
DE MENEZES, M.H.T.	MATHEUS HENRIQUES TAVARES DE MENEZES	3422
DE MESQUITA, L.B.	LILIANE BATISTA DE MESQUITA	3311
DE MIRANDA, B.T.	BEATRIZ TOJA DE MIRANDA	2925
DE MIRANDA, B.T.	BEATRIZ TOJA DE MIRANDA	2930
DE MIRANDA, G.C.	GUSTAVO CABRAL DE MIRANDA	3131
DE MIRANDA, P.H.	PEDRO HENRIQUE DE MIRANDA	3059
DE MIRANDA, T.T.	TATIANA TEIXEIRA DE MIRANDA	2965
DE MORAES, A.	ALEXSANDER DE MORAES	2875
DE MORAES, A.	ALEXSANDER DE MORAES	3033
DE MORAES, A.	ALEXSANDER DE MORAES	3275
DE MORAES, B.P.T.	BIANCA PORTUGAL TAVARES DE MORAES	3467
DE MORAES, D.B.C.	DANIELLA BORSARIO CIRENA DE MORAES	3062
DE MORAES, J.A.	JOÃO ALFREDO DE MORAES	2925
DE MORAES, L.M.P.	LIDIA MARIA PEPE DE MORAES	3152
DE MORAIS, F.R.	FABIANA ROSSETTO DE MORAIS	3285

DE MOURA, B.I.K.	BÁRBARA ISABEL KLEIN DE MOURA	3117
DE MOURA, B.I.K.	BÁRBARA ISABEL KLEIN DE MOURA	3368
DE MOURA, G.S.	GUILHERME SANTANA DE MOURA	3010
DE MOURA, P.M.M.F.	PATRICIA MUNIZ MENDES FREIRE DE MOURA	2833
DE MOURA, R.G.	RENAN GARCIA DE MOURA	3059
DE MOURA, R.G.	RENAN GARCIA DE MOURA	3123
DE MOURA, R.G.	RENAN GARCIA DE MOURA	3403
DE MOURA, R.G.	RENAN GARCIA DE MOURA	3483
DE MOURA, R.G.	RENAN GARCIA DE MOURA	3489
DE MOURA, V.E.	VICTORIA ELIZABETH DE MOURA	3314
DE MOURA, V.E.	VICTORIA ELIZABETH DE MOURA	3364
DE OLIVEIRA FILHO, V.A.	VIRLÂNIO ALVES DE OLIVEIRA FILHO	3034
DE OLIVEIRA, A.C.D.S.C.	ANA CAROLINA DE SIQUEIRA COUTO DE OLIVEIRA	3422
DE OLIVEIRA, A.C.D.S.C.	ANA CAROLINA DE SIQUEIRA COUTO DE OLIVEIRA	3478
DE OLIVEIRA, A.C.P.	ANTÔNIO CARLOS PINHEIRO DE OLIVEIRA	2685
DE OLIVEIRA, A.C.P.	ANTÔNIO CARLOS PINHEIRO DE OLIVEIRA	2847
DE OLIVEIRA, A.J.	ADRIANA JACAUNA DE OLIVEIRA	3026
DE OLIVEIRA, A.L.	ARYADNE LYRIO DE OLIVEIRA	3059
DE OLIVEIRA, A.L.L.	ANA LÍGIA LEANDRINI DE OLIVEIRA	2900
DE OLIVEIRA, A.P.L.	ANA PAULA LIGEIRO DE OLIVEIRA	2939
DE OLIVEIRA, B.	BIANCA DE OLIVEIRA	3276
DE OLIVEIRA, C.I.	CAMILA INDIANI DE OLIVEIRA	2779
DE OLIVEIRA, C.I.	CAMILA INDIANI DE OLIVEIRA	3210
DE OLIVEIRA, C.J.F.	CARLO JOSÉ FREIRE DE OLIVEIRA	2705
DE OLIVEIRA, C.J.F.	CARLO JOSÉ FREIRE DE OLIVEIRA	3454
DE OLIVEIRA, C.J.F.	CARLO JOSÉ FREIRE DE OLIVEIRA	3486
DE OLIVEIRA, C.J.F.	CARLO JOSÉ FREIRE DE OLIVEIRA	3493
DE OLIVEIRA, C.J.F.	CARLO JOSÉ FREIRE DE OLIVEIRA	3495
DE OLIVEIRA, C.V.	CAROLINE VITÓRIA DE OLIVEIRA	3333
DE OLIVEIRA, C.V.	CAROLINE VITÓRIA DE OLIVEIRA	3415
DE OLIVEIRA, D.M.	DOUGLAS MATHIAS DE OLIVEIRA	3426
DE OLIVEIRA, E.C.	ELAINE CARVALHO DE OLIVEIRA	2775
DE OLIVEIRA, E.C.	ELAINE CARVALHO DE OLIVEIRA	3156
DE OLIVEIRA, E.C.	ELAINE CARVALHO DE OLIVEIRA	3250
DE OLIVEIRA, E.C.	ELAINE CARVALHO DE OLIVEIRA	3298
DE OLIVEIRA, E.C.	ELAINE CARVALHO DE OLIVEIRA	3464
DE OLIVEIRA, E.C.M.	ELIDA CRISTINA MONTEIRO DE OLIVEIRA	2695
DE OLIVEIRA, E.C.M.	ELIDA CRISTINA MONTEIRO DE OLIVEIRA	2696
DE OLIVEIRA, E.C.M.	ELIDA CRISTINA MONTEIRO DE OLIVEIRA	2697
DE OLIVEIRA, E.C.M.	ELIDA CRISTINA MONTEIRO DE OLIVEIRA	2700
DE OLIVEIRA, E.C.M.	ELIDA CRISTINA MONTEIRO DE OLIVEIRA	2703
DE OLIVEIRA, E.C.M.	ELIDA CRISTINA MONTEIRO DE OLIVEIRA	2704
DE OLIVEIRA, E.C.P.	ELAINE CRISTINA PACHECO DE OLIVEIRA	3357
DE OLIVEIRA, E.C.P.	ELAINE CRISTINA PACHECO DE OLIVEIRA	3412
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	2801
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	2813
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	2822
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	2874
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	2889
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	2911
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	2966
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	3076
DE OLIVEIRA, E.E.	ERICK ESTEVES DE OLIVEIRA	3116
DE OLIVEIRA, E.M.	EVELYN MACIEL DE OLIVEIRA	2816
DE OLIVEIRA, E.M.	EVELYN MACIEL DE OLIVEIRA	2998
DE OLIVEIRA, E.Y.C.	ESTELA YASMIN COELHO DE OLIVEIRA	3323
DE OLIVEIRA, F.A.	FERNANDA ALMEIDA DE OLIVEIRA	3269
DE OLIVEIRA, F.A.	FERNANDA ALMEIDA DE OLIVEIRA	3270
DE OLIVEIRA, F.D.A.P.	FABIANE DOS ANJOS PENA DE OLIVEIRA	3192

DE OLIVEIRA, G.	GABRIELA DE OLIVEIRA	2667
DE OLIVEIRA, G.	GABRIELA DE OLIVEIRA	2669
DE OLIVEIRA, G.C.	GUSTAVO CAMARINHA DE OLIVEIRA	2828
DE OLIVEIRA, G.P.C.	GUSTAVO PEREIRA CARDOSO DE OLIVEIRA	2636
DE OLIVEIRA, G.P.C.	GUSTAVO PEREIRA CARDOSO DE OLIVEIRA	2639
DE OLIVEIRA, I.M.	ISADORA MAFRA DE OLIVEIRA	3401
DE OLIVEIRA, I.M.	ISADORA MAFRA DE OLIVEIRA	3421
DE OLIVEIRA, I.M.	ISADORA MAFRA DE OLIVEIRA	3465
DE OLIVEIRA, I.P.	ISABELA PENTERICHE DE OLIVEIRA	3002
DE OLIVEIRA, J.	JOSEANA DE OLIVEIRA	2914
DE OLIVEIRA, J.C.G.	JOYCE CRISTINA GUIMARÃES DE OLIVEIRA	2738
DE OLIVEIRA, J.C.G.	JOYCE CRISTINA GUIMARÃES DE OLIVEIRA	3418
DE OLIVEIRA, J.G.	JAQUELLINE GERMANO DE OLIVEIRA	3294
DE OLIVEIRA, J.L.	JADIVAN LEITE DE OLIVEIRA	3446
DE OLIVEIRA, J.L.	JORGETE LOGULLO DE OLIVEIRA	3463
DE OLIVEIRA, J.L.	JULIA LOPES DE OLIVEIRA	3146
DE OLIVEIRA, J.P.D.L.	JOÃO PEDRO DE LIMA DE OLIVEIRA	3394
DE OLIVEIRA, J.P.D.L.	JOÃO PEDRO DE LIMA DE OLIVEIRA	3457
DE OLIVEIRA, J.S.	JAMIL SILVANO DE OLIVEIRA	2676
DE OLIVEIRA, J.S.	JAMIL SILVANO DE OLIVEIRA	2684
DE OLIVEIRA, L.	LAUDICEIA DE OLIVEIRA	3326
DE OLIVEIRA, L.B.	LETÍCIA BERNARDES DE OLIVEIRA	2777
DE OLIVEIRA, L.D.	LETICIA DONAIRE DE OLIVEIRA	2934
DE OLIVEIRA, L.G.	LEANDRO GONZAGA DE OLIVEIRA	2661
DE OLIVEIRA, L.G.	LETÍCIA GOMES DE OLIVEIRA	3081
DE OLIVEIRA, L.G.	LETÍCIA GOMES DE OLIVEIRA	3082
DE OLIVEIRA, L.G.	LETÍCIA GOMES DE OLIVEIRA	3102
DE OLIVEIRA, L.G.	LETÍCIA GOMES DE OLIVEIRA	3147
DE OLIVEIRA, L.G.	LETÍCIA GOMES DE OLIVEIRA	3162
DE OLIVEIRA, L.G.	LETÍCIA GOMES DE OLIVEIRA	3213
DE OLIVEIRA, L.M.G.	LETÍCIA MORELLATO GARCIA DE OLIVEIRA	2965
DE OLIVEIRA, L.R.C.	LARISSA RAGOZO CARDOSO DE OLIVEIRA	2985
DE OLIVEIRA, L.R.C.	LARISSA RAGOZO CARDOSO DE OLIVEIRA	3222
DE OLIVEIRA, L.S.	LUANA SANTOS DE OLIVEIRA	2893
DE OLIVEIRA, L.S.	LUANA SANTOS DE OLIVEIRA	2940
DE OLIVEIRA, L.S.	LUCIANA SOUZA DE OLIVEIRA	2981
DE OLIVEIRA, L.S.	LUCIANA SOUZA DE OLIVEIRA	2993
DE OLIVEIRA, M.A.	MARIA APARECIDA DE OLIVEIRA	2894
DE OLIVEIRA, M.A.	MATHEUS ATELLA DE OLIVEIRA	3417
DE OLIVEIRA, M.C.	MARIANA CAVALCANTE DE OLIVEIRA	2742
DE OLIVEIRA, M.D.A.	MIGUEL DE ABREU DE OLIVEIRA	3387
DE OLIVEIRA, M.D.A.	MIGUEL DE ABREU DE OLIVEIRA	3391
DE OLIVEIRA, M.F.	MATHEUS FERNANDES DE OLIVEIRA	2975
DE OLIVEIRA, M.F.	MATHEUS FERNANDES DE OLIVEIRA	3143
DE OLIVEIRA, M.F.A.	MARCOS FELIPE ANDRADE DE OLIVEIRA	2656
DE OLIVEIRA, M.F.A.	MARCOS FELIPE ANDRADE DE OLIVEIRA	2665
DE OLIVEIRA, M.F.A.	MARCOS FELIPE ANDRADE DE OLIVEIRA	2676
DE OLIVEIRA, M.F.C.	MAIARA FONSECA CUPERTINO DE OLIVEIRA	3032
DE OLIVEIRA, P.A.	PEDRO AUGUSTO DE OLIVEIRA	2657
DE OLIVEIRA, P.E.M.	PEDRO ENRICO MARTIN DE OLIVEIRA	2866
DE OLIVEIRA, P.M.N.	PATRICIA MOUTA NUNES DE OLIVEIRA	3184
DE OLIVEIRA, P.M.N.	PATRICIA MOUTA NUNES DE OLIVEIRA	3243
DE OLIVEIRA, P.M.N.	PATRICIA MOUTA NUNES DE OLIVEIRA	3249
DE OLIVEIRA, P.M.N.	PATRICIA MOUTA NUNES DE OLIVEIRA	3411
DE OLIVEIRA, R.C.	REGIANE COSTA DE OLIVEIRA	3383
DE OLIVEIRA, R.C.	REGIANE COSTA DE OLIVEIRA	3414
DE OLIVEIRA, R.M.P.	RAYSSA MARRONNE PEREIRA DE OLIVEIRA	2738
DE OLIVEIRA, R.P.	RODRIGO PEREIRA DE OLIVEIRA	3109
DE OLIVEIRA, R.S.	RAFAELA SCARDINI DE OLIVEIRA	2757

DE OLIVEIRA, S.	SARAH DE OLIVEIRA	2843
DE OLIVEIRA, S.	SARAH DE OLIVEIRA	3220
DE OLIVEIRA, S.N.R.V.	SAMARA NASCIMENTO REZENDE VITORIA DE OLIVEIRA	3399
DE OLIVEIRA, S.R.	SIRLEY ROCHA DE OLIVEIRA	3286
DE OLIVEIRA, T.B.	THIAGO BUENO DE OLIVEIRA	3317
DE OLIVEIRA, T.G.	TALITA GONÇALVES DE OLIVEIRA	2975
DE OLIVEIRA, T.G.	TALITA GONÇALVES DE OLIVEIRA	3143
DE OLIVEIRA, T.G.M.	THÉO GREMEN MIMARY DE OLIVEIRA	2854
DE OLIVEIRA, T.G.M.	THÉO GREMEN MIMARY DE OLIVEIRA	3040
DE OLIVEIRA, T.G.M.	THÉO GREMEN MIMARY DE OLIVEIRA	3221
DE OLIVEIRA, T.R.	TATIANE RODRIGUES DE OLIVEIRA	2950
DE OLIVEIRA, T.R.	TATIANE RODRIGUES DE OLIVEIRA	3007
DE OLIVEIRA, T.S.	THAIS SILVA DE OLIVEIRA	2814
DE OLIVEIRA, V.	VICTÓRIA DE OLIVEIRA	3139
DE OLIVEIRA, V.L.	VIVIAN LEITE DE OLIVEIRA	2928
DE OLIVEIRA, V.L.	VIVIAN LEITE DE OLIVEIRA	3093
DE OLIVEIRA, V.L.	VIVIAN LEITE DE OLIVEIRA	3101
DE OLIVEIRA, V.L.	VIVIAN LEITE DE OLIVEIRA	3140
DE OLIVEIRA, V.L.S.	VÍVIAN LOUISE SOARES DE OLIVEIRA	2846
DE PAIVA, K.L.R.	KAREN LETYCIA RODRIGUES DE PAIVA	2763
DE PAIVA, K.L.R.	KAREN LETYCIA RODRIGUES DE PAIVA	2764
DE PAIVA, L.S.	LUCIANA SOUZA DE PAIVA	2758
DE PAOLI, F.	FLÁVIA DE PAOLI	3458
DE PASCALI, D.J.	DÁRIO JESUS DE PASCALI	3432
DE PAULA, A.	ALINE DE PAULA	3479
DE PAULA, C.	CRISTINA DE PAULA	3305
DE PAULA, C.	CRISTINA DE PAULA	3334
DE PAULA, C.	CRISTINA DE PAULA	3433
DE PAULA, L.S.M.	LORRAINE STEFANIE MOREIRA DE PAULA	3100
DE PAULA, M.M.L.	MARCELO MIRANDA LIMA DE PAULA	3247
DE PAULA, M.M.L.	MARCELO MIRANDA LIMA DE PAULA	3273
DE PAULA, M.M.L.	MARCELO MIRANDA LIMA DE PAULA	3456
DE PAULA, N.A.	NATÁLIA APARECIDA DE PAULA	3401
DE PINHO, A.M.V.F.	ARTHUR MIGLIATTI VETORAZZI FERREIRA DE PINHO	2665
DE PINHO, A.M.V.F.	ARTHUR MIGLIATTI VETORAZZI FERREIRA DE PINHO	2676
DE PONTES, L.G.	LETICIA GOMES DE PONTES	2670
DE PONTES, L.G.	LETICIA GOMES DE PONTES	2691
DE PONTES, L.G.	LETICIA GOMES DE PONTES	2692
DE PONTES, P.H.P.C.	PEDRO HENRIQUE PAIVA CAMELO DE PONTES	2837
DE PONTES, P.H.P.C.	PEDRO HENRIQUE PAIVA CAMELO DE PONTES	3015
DE PONTES, P.H.P.C.	PEDRO HENRIQUE PAIVA CAMELO DE PONTES	3017
DE QUADROS, A.U.	ANDREZA URBA DE QUADROS	2959
DE QUADROS, A.U.	ANDREZA URBA DE QUADROS	3016
DE QUEIROZ, A.M.	AMANDA MARIA DE QUEIROZ	2765
DE QUEIROZ, A.M.	AMANDA MARIA DE QUEIROZ	2877
DE QUEIROZ, A.M.	ARIADNE MENDES DE QUEIROZ	2868
DE QUEIROZ, L.H.	LETICIA HAGALE DE QUEIROZ	3336
DE QUEIROZ, L.T.	LUCAS TAVARES DE QUEIROZ	3050
DE QUEIROZ, N.M.G.P.	NINA MARI GUAL PIMENTA DE QUEIROZ	2781
DE QUEIROZ, N.M.G.P.	NINA MARI GUAL PIMENTA DE QUEIROZ	2981
DE ROSSI, T.	TATIANE DE ROSSI	2733
DE SÁ, A.B.M.	ANA BEATRIZ MIRANDA DE SÁ	3110
DE SÁ, B.D.S.	BIANCA DOS SANTOS DE SÁ	2823
DE SÁ, B.D.S.	BIANCA DOS SANTOS DE SÁ	3363
DE SÁ, F.M.C.	FERNANDA MIRANDA CALIANI DE SÁ	2749
DE SÁ, K.	KEYLA DE SÁ	3465
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	2897
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	2904
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	3099

DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	3132
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	3138
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	3195
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	3238
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	3241
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	3256
DE SÁ, M.C.M.D.M.A.	MARIA CLARA MARTINS DE MATTOS AMORIM DE SÁ	3261
DE SÁ, N.B.R.	NATHALIA BEATRIZ RAMOS DE SÁ	3000
DE SÁ, N.B.R.	NATHALIA BEATRIZ RAMOS DE SÁ	3105
DE SÁ, N.B.R.	NATHALIA BEATRIZ RAMOS DE SÁ	3240
DE SÁ, N.B.R.	NATHALIA BEATRIZ RAMOS DE SÁ	3260
DE SÁ, R.S.	RAÍSA SALES DE SÁ	3317
DE SALES, B.L.	BRAD LIRA DE SALES	3383
DE SALES, J.L.	JÚLIA LOPES DE SALES	3250
DE SALES, J.L.	JÚLIA LOPES DE SALES	3298
DE SALES, J.L.	JÚLIA LOPES DE SALES	3464
DE SALLES, É.M.	ÉRIKA MACHADO DE SALLES	2855
DE SALLES, É.M.	ÉRIKA MACHADO DE SALLES	3040
DE SANTANA, M.B.R.	MARIA BORGES RABELO DE SANTANA	3291
DE SANTANA, M.B.R.	MARIA BORGES RABELO DE SANTANA	3517
DE SANTANA, M.B.R.	MARINA BORGES RABELO DE SANTANA	2779
DE SANTANA, M.B.R.	MARINA BORGES RABELO DE SANTANA	3210
DE SANTANA, M.B.R.	MARINA BORGES RABELO DE SANTANA	3517
DE SANTIS, P.B.	PIETRA BURATTO DE SANTIS	3120
DE SIMONE, C.	CHIARA DE SIMONE	3329
DE SIMONE, S.G.	SALVATORE GIOVANNI DE SIMONE	3047
DE SIQUEIRA, L.H.	LUCIA HELENA DE SIQUEIRA	3360
DE SIQUEIRA, M.K.	MIRIAN KRYSTEL DE SIQUEIRA	2944
DE SIQUEIRA, P.F.	PEDRO FERREIRA DE SIQUEIRA	3043
DE SIQUEIRA, P.F.	PEDRO FERREIRA DE SIQUEIRA	3063
DE SOUSA FILHO, I.A.	IDIO ALVES DE SOUSA FILHO	2936
DE SOUSA, E.S.C.	ELIS SANTANA CURVELO DE SOUSA	2871
DE SOUSA, E.S.C.	ELIS SANTANA CURVELO DE SOUSA	2887
DE SOUSA, E.S.C.	ELIS SANTANA CURVELO DE SOUSA	2910
DE SOUSA, E.S.C.	ELIS SANTANA CURVELO DE SOUSA	3025
DE SOUSA, L.P.	LIRLÂNDIA PIRES DE SOUSA	2661
DE SOUSA, L.P.	LIRLÂNDIA PIRES DE SOUSA	2669
DE SOUZA, A.C.L.	ANA CLARA LEAL DE SOUZA	2871
DE SOUZA, A.C.L.	ANA CLARA LEAL DE SOUZA	2887
DE SOUZA, A.C.L.	ANA CLARA LEAL DE SOUZA	2910
DE SOUZA, A.C.L.	ANA CLARA LEAL DE SOUZA	3025
DE SOUZA, A.F.N.	ANA FLAVIA NUNES DE SOUZA	3314
DE SOUZA, A.F.N.	ANA FLAVIA NUNES DE SOUZA	3364
DE SOUZA, A.P.D.	ANA PAULA DUARTE DE SOUZA	3321
DE SOUZA, A.P.D.	ANA PAULA DUARTE DE SOUZA	3435
DE SOUZA, A.P.D.	ANA PAULA DUARTE DE SOUZA	3455
DE SOUZA, B.R.	BRUNO REZENDE DE SOUZA	2702
DE SOUZA, D.C.D.S.R.	DANILO CHAVES DA SILVA RAMOS DE SOUZA	2989
DE SOUZA, D.C.D.S.R.	DANILO CHAVES DA SILVA RAMOS DE SOUZA	3008
DE SOUZA, D.C.D.S.R.	DANILO CHAVES DA SILVA RAMOS DE SOUZA	3230
DE SOUZA, D.C.D.S.R.	DANILO CHAVES DA SILVA RAMOS DE SOUZA	3325
DE SOUZA, D.D.G.	DANIELLE DA GLÓRIA DE SOUZA	2657
DE SOUZA, D.D.G.	DANIELLE DA GLÓRIA DE SOUZA	2675
DE SOUZA, D.D.G.	DANIELLE DA GLÓRIA DE SOUZA	2685
DE SOUZA, D.D.G.	DANIELLE DA GLÓRIA DE SOUZA	3316
DE SOUZA, D.D.G.	DANIELLE DA GLÓRIA DE SOUZA	3434
DE SOUZA, D.D.G.	DANIELLE DA GLÓRIA DE SOUZA	3451
DE SOUZA, E.E.	EDMARCIA ELISA DE SOUZA	2740
DE SOUZA, E.E.	EDMARCIA ELISA DE SOUZA	2865

DE SOUZA, E.E.	EDMARCIA ELISA DE SOUZA	3149
DE SOUZA, E.K.	ELLEN KIARELY DE SOUZA	3257
DE SOUZA, E.R.	EMANUELL RODRIGUES DE SOUZA	2994
DE SOUZA, E.R.	EMANUELL RODRIGUES DE SOUZA	2996
DE SOUZA, E.R.	EMANUELL RODRIGUES DE SOUZA	3021
DE SOUZA, E.R.	EMANUELL RODRIGUES DE SOUZA	3035
DE SOUZA, F.N.	FERNANDO NOGUEIRA DE SOUZA	3010
DE SOUZA, F.N.	FERNANDO NOGUEIRA DE SOUZA	3281
DE SOUZA, G.B.	GABRIEL BRUM DE SOUZA	3352
DE SOUZA, G.S.	GLAUCIO SILVA DE SOUZA	3331
DE SOUZA, H.A.D.S.	HUGO AMORIM DOS SANTOS DE SOUZA	2743
DE SOUZA, H.A.D.S.	HUGO AMORIM DOS SANTOS DE SOUZA	2893
DE SOUZA, H.A.D.S.	HUGO AMORIM DOS SANTOS DE SOUZA	3050
DE SOUZA, H.R.	HYGOR RIBERIO DE SOUZA	3404
DE SOUZA, H.S.P.	HEITOR SIFFERT PEREIRA DE SOUZA	3086
DE SOUZA, H.S.P.	HEITOR SIFFERT PEREIRA DE SOUZA	3109
DE SOUZA, H.S.P.	HEITOR SIFFERT PEREIRA DE SOUZA	3166
DE SOUZA, H.S.P.	HEITOR SIFFERT PEREIRA DE SOUZA	3169
DE SOUZA, H.S.P.	HEITOR SIFFERT PEREIRA DE SOUZA	3306
DE SOUZA, I.A.	IZABELA APARECIDA DE SOUZA	2671
DE SOUZA, I.A.	IZABELA APARECIDA DE SOUZA	2681
DE SOUZA, I.A.	IZABELA APARECIDA DE SOUZA	2688
DE SOUZA, I.F.	INGRID FERREIRA DE SOUZA	3472
DE SOUZA, I.F.	ISADORA F. DE SOUZA	2763
DE SOUZA, I.F.	ISADORA F. DE SOUZA	2764
DE SOUZA, I.L.L.	ISADORA LUIZA LIMA DE SOUZA	3102
DE SOUZA, I.S.	ISABELLA SPADON DE SOUZA	3275
DE SOUZA, J.F.F.	JOSÉ FÁBIO FERREIRA DE SOUZA	3265
DE SOUZA, L.D.R.	LUANA DOS REIS DE SOUZA	3187
DE SOUZA, L.E.B.	LUCAS EDUARDO BOTELHO DE SOUZA	2861
DE SOUZA, L.E.B.	LUCAS EDUARDO BOTELHO DE SOUZA	2870
DE SOUZA, L.E.B.	LUCAS EDUARDO BOTELHO DE SOUZA	2959
DE SOUZA, L.E.B.	LUCAS EDUARDO BOTELHO DE SOUZA	2964
DE SOUZA, L.E.B.	LUCAS EDUARDO BOTELHO DE SOUZA	2969
DE SOUZA, L.E.B.	LUCAS EDUARDO BOTELHO DE SOUZA	3016
DE SOUZA, L.L.	LAURA LIBÂNIO DE SOUZA	2821
DE SOUZA, L.L.	LAURA LIBÂNIO DE SOUZA	2896
DE SOUZA, L.L.	LAURA LIBÂNIO DE SOUZA	3039
DE SOUZA, L.M.	LORENA MIRANDA DE SOUZA	2850
DE SOUZA, L.V.O.	LORENNA VICTÓRIA OLIVEIRA DE SOUZA	2727
DE SOUZA, M.	MARIANA DE SOUZA	3357
DE SOUZA, M.	MARIANA DE SOUZA	3412
DE SOUZA, M.A.	MATHEUS AJEJE DE SOUZA	2845
DE SOUZA, M.A.	MATHEUS AJEJE DE SOUZA	2848
DE SOUZA, M.E.R.	MARIA EDUARDA RODRIGUES DE SOUZA	2738
DE SOUZA, M.V.O.	MARIA VITÓRIA OLIVEIRA DE SOUZA	3468
DE SOUZA, N.	NOEMI DE SOUZA	3298
DE SOUZA, N.	NOEMI DE SOUZA	3315
DE SOUZA, N.	NOEMI DE SOUZA	3464
DE SOUZA, N.S.H.	NATÁLIA SATCHIKO HOJO DE SOUZA	2658
DE SOUZA, N.S.H.	NATÁLIA SATCHIKO HOJO DE SOUZA	2672
DE SOUZA, N.S.H.	NATÁLIA SATCHIKO HOJO DE SOUZA	2865
DE SOUZA, P.H.O.	PEDRO HENRIQUE OLIVEIRA DE SOUZA	3311
DE SOUZA, P.P.C.	PEDRO PAULO CHAVES DE SOUZA	3119
DE SOUZA, P.V.D.	PAULO VITOR DIAS DE SOUZA	3144
DE SOUZA, P.V.D.	PAULO VITOR DIAS DE SOUZA	3242
DE SOUZA, R.L.L.	RAFAELA LÚCIA LOPES DE SOUZA	3079
DE SOUZA, R.L.L.	RAFAELA LÚCIA LOPES DE SOUZA	3264
DE SOUZA, R.L.L.	RAFAELA LÚCIA LOPES DE SOUZA	3375

DE SOUZA, R.L.L.	RAFAELA LÚCIA LOPES DE SOUZA	3428
DE SOUZA, R.P.	RENAN PEDRA DE SOUZA	2781
DE SOUZA, R.Q.	RAFAEL QUEIROZ DE SOUZA	3191
DE SOUZA, R.Q.	RAFAEL QUEIROZ DE SOUZA	3229
DE SOUZA, R.Q.	RAFAEL QUEIROZ DE SOUZA	3235
DE SOUZA, R.S.	RAYSA SILVA DE SOUZA	2778
DE SOUZA, R.S.	RAYSA SILVA DE SOUZA	3154
DE SOUZA, R.S.D.	RENATA SOARES DIAS DE SOUZA	3311
DE SOUZA, R.V.O.	RACHEL V. O'NEILL DE SOUZA	2998
DE SOUZA, T.A.M.	TAÍS APARECIDA MATOZO DE SOUZA	2855
DE SOUZA, T.H.F.	THALLES HENRIQUE FARIA DE SOUZA	3246
DE SOUZA, T.M.	TATIANA MARIA DE SOUZA	3460
DE SOUZA, V.P.	VIVIANE PASSOS DE SOUZA	2813
DE SOUZA, V.P.	VIVIANE PASSOS DE SOUZA	2822
DE SOUZA, V.P.	VIVIANE PASSOS DE SOUZA	3116
DE SOUZA, V.P.	VIVIANE PASSOS DE SOUZA	3280
DE SOUZA, V.P.	VIVIANE PASSOS DE SOUZA	3302
DE SOUZA, Y.B.L.	YASMIN BRITO LEITE DE SOUZA	3328
DE SOUZA-SILVA, G.A.	GUILHERME ANTONIO DE SOUZA-SILVA	2928
DE SOUZA-SILVA, G.A.	GUILHERME ANTONIO DE SOUZA-SILVA	2950
DE VASCONCELOS, A.G.P.	ANA GABRIELA PEREIRA DE VASCONCELOS	2920
DE VASCONCELOS, Z.F.M.	ZILTON FARIAS MEIRA DE VASCONCELOS	3065
DE VASCONCELOS, Z.F.M.	ZILTON FARIAS MEIRA DE VASCONCELOS	3137
DE VASCONCELOS, Z.F.M.	ZILTON FARIAS MEIRA DE VASCONCELOS	3174
DE VASCONCELOS, Z.F.M.	ZILTON FARIAS MEIRA DE VASCONCELOS	3184
DE VASCONCELOS, Z.F.M.	ZILTON FARIAS MEIRA DE VASCONCELOS	3249
DE VASCONCELOS, Z.F.M.	ZILTON FARIAS MEIRA DE VASCONCELOS	3268
DE VASCONCELOS, Z.F.M.	ZILTON FARIAS MEIRA DE VASCONCELOS	3371
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	2738
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	2862
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	2912
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	2918
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	2936
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	2948
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	3232
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	3403
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	3418
DECOTE-RICARDO, D.	DEBORA DECOTE-RICARDO	3484
DEFELICIBUS, A.	ALEXANDRE DEFELICIBUS	3314
DEFELICIBUS, A.	ALEXANDRE DEFELICIBUS	3364
DE-FREITAS-JUNIOR, J.C.M.	JULIO CESAR MADUREIRA DE-FREITAS-JUNIOR	2925
DELATORRE, E.O.	EDSON OLIVEIRA DELATORRE	3409
DELECHIAVE, D.	DANIELA DELECHIAVE	2761
DELECHIAVE, D.	DANIELA DELECHIAVE	2791
DELECHIAVE, D.	DANIELA DELECHIAVE	2827
DELECHIAVE, D.	DANIELA DELECHIAVE	2841
DELECHIAVE, D.	DANIELA DELECHIAVE	3094
DELECHIAVE, D.	DANIELA DELECHIAVE	3171
DELECHIAVE, D.	DANIELA DELECHIAVE	3309
DELGADO, M.V.B.	MILLENA VICENTE BARBOSA DELGADO	3047
DENADAI, M.B.	MARINA BONIFÁCIO DENADAI	2914
DENADAI, M.B.	MARINA BONIFÁCIO DENADAI	3513
DENISET, J.	JUSTIN DENISET	2853
DE-OLIVEIRA-PINTO, L.M.	LUZIA MARIA DE-OLIVEIRA-PINTO	2742
DERMARGOS, A.	ALEXANDRE DERMARGOS	2954
DERMARGOS, E.H.	ELAINE HATANAKA DERMARGOS	2954
DESIDÉRIO, C.S.	CHAMBERTTAN SOUZA DESIDÉRIO	2705
DESIDÉRIO, C.S.	CHAMBERTTAN SOUZA DESIDÉRIO	3454
DESIDÉRIO, C.S.	CHAMBERTTAN SOUZA DESIDÉRIO	3486

DESIDÉRIO, C.S.	CHAMBERTTAN SOUZA DESIDÉRIO	3493
DESIDÉRIO, C.S.	CHAMBERTTAN SOUZA DESIDÉRIO	3495
DESIDÉRIO, C.S.	CHAMBERTTAN SOUZA DESIDÉRIO	3497
DESIDÉRIO, C.S.	CHAMBERTTAN SOUZA DESIDÉRIO	3516
DETONI, M.A.A.	MARCELLA ALMEIDA AZEVEDO DETONI	3072
DEZEN, A.L.O.	ANA LAURA ORTEGA DEZEN	3382
DHILLON, P.	POONAM DHILLON	3506
DIAS, A.A.	ANDRÉ ALVES DIAS	3228
DIAS, A.A.	ANDRÉ ALVES DIAS	3466
DIAS, A.D.A.S.	ADRIANE DE ASSIS SILVA DIAS	2675
DIAS, A.G.	AYRES GUIMARÃES DIAS	2891
DIAS, A.G.D.S.	ALEXANDRE GUILHERME DA SILVA DIAS	3522
DIAS, A.S.L.	ANGÉLICA SAMER LALLO DIAS	2686
DIAS, A.S.L.	ANGÉLICA SAMER LALLO DIAS	2988
DIAS, B.B.	BEATRIZ BELMIRO DIAS	2954
DIAS, L.C.C.	LAURA CARDOSO CORRÊA DIAS	2670
DIAS, L.C.C.	LAURA CARDOSO CORRÊA DIAS	2687
DIAS, L.C.C.	LAURA CARDOSO CORRÊA DIAS	2690
DIAS, L.C.C.	LAURA CARDOSO CORRÊA DIAS	2692
DIAS, L.M.	LARISSA MOREIRA DIAS	2869
DIAS, M.D.J.	MARIANA DE JESUS DIAS	3229
DIAS, M.V.	MARIANA VIEIRA DIAS	2685
DIAS, M.V.	MARIANA VIEIRA DIAS	3316
DIAS, N.L.S.	NATHÁLIA L S DIAS	2833
DIAS, P.M.	PRISCILA MARTINS DIAS	3087
DIAS, S.D.S.G.	SUELEN DA SILVA GOMES DIAS	3426
DIAS, S.D.S.G.	SUELEN DA SILVA GOMES DIAS	3480
DIAS, S.D.S.G.	SUELEN DA SILVA GOMES DIAS	3482
DIAS, S.D.S.G.	SUELEN DA SILVA GOMES DIAS	3490
DIAS, T.D.S.	TAYNÁ DA SILVA DIAS	2936
DIAS, T.D.S.	TAYNÁ DA SILVA DIAS	2948
DIAS, W.B.	WAGNER BARBOSA DIAS	3463
DIAS-MELICIO, L.A.	LUCIANE ALARCAO DIAS-MELICIO	2952
DIAS-MELICIO, L.A.	LUCIANE ALARCAO DIAS-MELICIO	3382
DIAVAO, J.	JACIARA DIAVAO	2906
DIAVAO, J.	JACIARA DIAVAO	3096
DIAVAO, J.	JACIARA DIAVAO	3450
DÍAZ, Á.	ÁLVARO DÍAZ	2820
DIAZ, B.L.	BRUNO LOURENÇO DIAZ	2758
DIAZ, B.L.	BRUNO LOURENÇO DIAZ	3014
DIAZ, B.L.	BRUNO LOURENÇO DIAZ	3072
DIAZ, B.L.	BRUNO LOURENÇO DIAZ	3274
DIAZ, B.L.	BRUNO LOURENÇO DIAZ	3328
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3116
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3248
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3273
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3280
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3287
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3302
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3331
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3340
DIB, P.R.B.	PAULA RIBEIRO BRAGA DIB	3456
DIEHL, C.	CAMILA DIEHL	3353
DIERICK, E.	EVELIEN DIERICK	3115
DINIZ, L.N.A.	LARISSA NADUR ARANTES DINIZ	2685
DINIZ, L.N.A.	LARISSA NADUR ARANTES DINIZ	3316
DINIZ, L.N.A.	LARISSA NADUR ARANTES DINIZ	3451
DINIZ, L.R.V.	LETÍCIA RODRIGUES VILAS DINIZ	3463
DINIZ, M.D.O.	MARIANA DE OLIVEIRA DINIZ	2844

DIVERT, T.	TATYANA DIVERT	3115
DIZ, M.D.P.E.	MARIA DEL PILAR ESTEVEZ DIZ	2774
DL, O.	OLIVEIRA DL	3107
DO ALMO, M.M.	MANUELA MARAGNO DO ALMO	3152
DO AMARAL, J.B.	JÔNATAS BUSSADOR DO AMARAL	3419
DO AMARAL, L.P.	LOHANNA PALHINHA DO AMARAL	3331
DO AMARAL, R.V.	RAISSA VIEIRA DO AMARAL	3075
DO AMARAL, R.V.	RAISSA VIEIRA DO AMARAL	3520
DO ARAÚJO, E.C.S.	ERIKA CRHISTINA SANTOS DO ARAÚJO	2983
DO BRASIL, P.E.A.A.	PEDRO EMMANUEL ALVARENGA AMERICANO DO BRASIL	3105
DO CANTO, L.V.	LUIZE VELHO DO CANTO	3118
DO CARMO, Í.A.R.	ÍCARO ANDRADE RODRIGUES DO CARMO	3300
DO CARMO, R.F.	RODRIGO FELICIANO DO CARMO	2833
DO COUTO, M.S.	MATHEUS SILVA DO COUTO	3336
DO NASCIMENTO, A.H.	ALEXANDRO HOLANDA DO NASCIMENTO	2900
DO NASCIMENTO, A.L.C.	ARNALDO LEVINO COLARES DO NASCIMENTO	3159
DO NASCIMENTO, E.M.	EVELYN MENDES DO NASCIMENTO	2728
DO NASCIMENTO, G.P.	GABRIEL PASQUARELLI DO NASCIMENTO	3069
DO NASCIMENTO, I.V.	IVANA VENTURA DO NASCIMENTO	3095
DO NASCIMENTO, L.A.	LARA AREIAS DO NASCIMENTO	3164
DO NASCIMENTO, M.D.	MELISSA DIAS DO NASCIMENTO	3102
DO NASCIMENTO, M.D.	MELISSA DIAS DO NASCIMENTO	3147
DO NASCIMENTO, M.D.	MELISSA DIAS DO NASCIMENTO	3213
DO NASCIMENTO, M.L.C.	MARIA LAURA COSTA DO NASCIMENTO	2947
DO NASCIMENTO, R.L.L.D.	RYAN LUCAS LIMA DUARTE DO NASCIMENTO	3240
DO NASCIMENTO, R.L.L.D.	RYAN LUCAS LIMA DUARTE DO NASCIMENTO	3260
DO NASCIMENTO, R.M.C.	REBECCA MARTINS CADIMO DO NASCIMENTO	3446
DO NASCIMENTO, R.S.	ROGÉRIO SILVA DO NASCIMENTO	2989
DO NASCIMENTO, R.S.	ROGÉRIO SILVA DO NASCIMENTO	3008
DO NASCIMENTO, R.S.	ROGÉRIO SILVA DO NASCIMENTO	3230
DO NASCIMENTO, S.R.	SAMUEL RAMOS DO NASCIMENTO	2989
DO PATROCINIO, A.B.	ANDRESSA BARBAN DO PATROCINIO	3428
DO ROSÁRIO, N.F.	NATALIA FONSECA DO ROSÁRIO	2816
DO ROSÁRIO, N.F.	NATALIA FONSECA DO ROSÁRIO	2998
DO SACRAMENTO, P.M.	PRISCILA MENDONÇA DO SACRAMENTO	3062
DO VALLE, C.Z.	CAMILA ZAVERUCHA DO VALLE	3036
DOMINGUES, R.	ROBERT DOMINGUES	2906
DOMINGUES, R.	ROBERT DOMINGUES	3096
DOMINGUES, R.	ROBERT DOMINGUES	3450
DOMINGUES, V.T.	VITOR TORQUATO DOMINGUES	2749
DOMINGUES, V.T.	VITOR TORQUATO DOMINGUES	2934
DOMINGUEZ, A.C.	ALEJANDRO CORREA DOMINGUEZ	2860
DOMONT, G.B.	GILBERTO BARBOSA DOMONT	2810
DONASSOLO, R.A.	RAFAEL AMARAL DONASSOLO	2743
DOORES, K.	KATIE DOORES	2978
DORETTO-SILVA, L.	LORENA DORETTO-SILVA	2917
DORNELES, G.P.	GILSON PIRES DORNELES	2826
DORO, D.	DANIEL DORO	2658
DOS REIS, J.G.A.C.	JORDANA GRAZZIELA ALVES COELHO DOS REIS	2674
DOS REIS, J.G.A.C.	JORDANA GRAZZIELA ALVES COELHO DOS REIS	2687
DOS REIS, J.G.A.C.	JORDANA GRAZZIELA ALVES COELHO DOS REIS	2690
DOS REIS, J.G.A.C.	JORDANA GRAZZIELA ALVES COELHO DOS REIS	2714
DOS REIS, J.G.A.C.	JORDANA GRAZZIELA ALVES COELHO DOS REIS	3251
DOS REIS, J.G.A.C.	JORDANA GRAZZIELA ALVES COELHO DOS REIS	3294
DOS REIS, L.B.	LÍVIA BITTENCOURT DOS REIS	3203
DOS REIS, L.M.	LARISSA MENEZES DOS REIS	2832
DOS REIS, L.M.	LARISSA MENEZES DOS REIS	2882
DOS REIS, L.M.	LARISSA MENEZES DOS REIS	2920
DOS REIS, L.M.	LARISSA MENEZES DOS REIS	3034

DOS REIS, L.M.	LARISSA MENEZES DOS REIS	3487
DOS REIS, R.H.	RAINÁ HONÓRIO DOS REIS	3258
DOS REIS, S.A.	SABRINA ALVES DOS REIS	3135
DOS REIS, S.A.	SABRINA ALVES DOS REIS	3142
DOS REIS, S.A.	SABRINA ALVES DOS REIS	3332
DOS REIS, S.A.	SABRINA ALVES DOS REIS	3385
DOS REIS, T.C.B.	TANIA CAROLINA BRAGA DOS REIS	2855
DOS REIS, V.P.	VALDISON PEREIRA DOS REIS	2971
DOS SANTOS, A.C.C.	ANA CAROLINA CAMPOS DOS SANTOS	3070
DOS SANTOS, A.C.C.	ANA CAROLINA CAMPOS DOS SANTOS	3109
DOS SANTOS, A.C.P.M.	ANNA CLARA PAIVA MENEZES DOS SANTOS	2657
DOS SANTOS, A.C.P.M.	ANNA CLARA PAIVA MENEZES DOS SANTOS	2685
DOS SANTOS, B.C.	BEATRIZ CERQUEIRA DOS SANTOS	3341
DOS SANTOS, B.G.D.F.	BRUNO GUIMARAES DA FONSECA DOS SANTOS	3282
DOS SANTOS, B.V.	BIANCA VIEIRA DOS SANTOS	3515
DOS SANTOS, C.A.C.	CÍCERA ALEXSANDRA COSTA DOS SANTOS	3421
DOS SANTOS, C.D.O.	CAMILLA DE OLIVEIRA DOS SANTOS	3240
DOS SANTOS, C.D.O.	CAMILLA DE OLIVEIRA DOS SANTOS	3260
DOS SANTOS, C.R.	CAROLINE REIS DOS SANTOS	3065
DOS SANTOS, C.R.	CAROLINE REIS DOS SANTOS	3371
DOS SANTOS, C.V.	CINTIA VIEIRA DOS SANTOS	3269
DOS SANTOS, D.	DOUGLAS DOS SANTOS	3492
DOS SANTOS, D.C.	DANIELA COELHO DOS SANTOS	2755
DOS SANTOS, D.C.M.	DAMIAO CARLOS MORAES DOS SANTOS	3521
DOS SANTOS, D.R.L.	DEBORA REGINA LOPES DOS SANTOS	2918
DOS SANTOS, E.A.S.	EDUARDO AUGUSTO SARTORI DOS SANTOS	2670
DOS SANTOS, E.D.P.P.	ELAINE DE PAIVA PEREIRA DOS SANTOS	3494
DOS SANTOS, F.A.	FELIPE ARAUJO DOS SANTOS	3323
DOS SANTOS, G.M.	GUILHERME MELO DOS SANTOS	3173
DOS SANTOS, H.F.	HENRIQUE FRAGOSO DOS SANTOS	2830
DOS SANTOS, H.F.	HENRIQUE FRAGOSO DOS SANTOS	2859
DOS SANTOS, H.F.	HENRIQUE FRAGOSO DOS SANTOS	2888
DOS SANTOS, H.P.	HOZANY PRAXEDES DOS SANTOS	3346
DOS SANTOS, I.M.F.	IDÁLIA MARIA FERREIRA DOS SANTOS	2738
DOS SANTOS, I.M.F.	IDÁLIA MARIA FERREIRA DOS SANTOS	2787
DOS SANTOS, J.C.	JULIANA COSTA DOS SANTOS	3445
DOS SANTOS, J.P.	JULIANNA PEREIRA DOS SANTOS	2827
DOS SANTOS, J.P.	JULIANNA PEREIRA DOS SANTOS	2841
DOS SANTOS, J.P.	JULIANNA PEREIRA DOS SANTOS	3094
DOS SANTOS, J.P.R.S.	JOÃO PEDRO RIBEIRO SOARES DOS SANTOS	3247
DOS SANTOS, J.P.R.S.	JOÃO PEDRO RIBEIRO SOARES DOS SANTOS	3273
DOS SANTOS, J.P.R.S.	JOÃO PEDRO RIBEIRO SOARES DOS SANTOS	3456
DOS SANTOS, J.R.	JONATHAS RODRIGO DOS SANTOS	3269
DOS SANTOS, L.B.R.	LUANA BARBOSA RODRIGUES DOS SANTOS	2990
DOS SANTOS, L.B.R.	LUANA BARBOSA RODRIGUES DOS SANTOS	3020
DOS SANTOS, L.D.R.	LARISSA DIAS RIBEIRO DOS SANTOS	3209
DOS SANTOS, L.I.	LUARA ISABELA DOS SANTOS	2638
DOS SANTOS, L.I.	LUARA ISABELA DOS SANTOS	2713
DOS SANTOS, L.I.	LUARA ISABELA DOS SANTOS	2715
DOS SANTOS, L.M.	LILIANE MARTINS DOS SANTOS	2655
DOS SANTOS, L.M.	LILIANE MARTINS DOS SANTOS	2661
DOS SANTOS, L.M.	LILIANE MARTINS DOS SANTOS	2669
DOS SANTOS, L.M.P.	LAIZA MARTINS PEIXOTO DOS SANTOS	2630
DOS SANTOS, M.D.R.Z.	MATEUS DOS REIS ZUBA DOS SANTOS	3271
DOS SANTOS, M.D.R.Z.	MATEUS DOS REIS ZUBA DOS SANTOS	3367
DOS SANTOS, M.H.	MATHEUS HENRIQUE DOS SANTOS	2802
DOS SANTOS, M.M.	MURILO MARQUES DOS SANTOS	3381
DOS SANTOS, N.G.P.	NATALIA GABRIELLY PEREIRA DOS SANTOS	3326
DOS SANTOS, N.S.A.	NAILMA SILVA APRIGIO DOS SANTOS	2651

DOS SANTOS, P.D.M.A.	PABLO DE MELO ANDRADE DOS SANTOS	3015
DOS SANTOS, P.D.M.A.	PABLO DE MELO ANDRADE DOS SANTOS	3055
DOS SANTOS, P.D.M.A.	PABLO DE MELO ANDRADE DOS SANTOS	3279
DOS SANTOS, P.D.M.A.	PABLO DE MELO ANDRADE DOS SANTOS	3308
DOS SANTOS, P.D.M.A.	PABLO DE MELO ANDRADE DOS SANTOS	3310
DOS SANTOS, P.D.M.A.	PABLO DE MELO ANDRADE DOS SANTOS	3361
DOS SANTOS, R.M.	RAMON MENDES DOS SANTOS	3282
DOS SANTOS, R.Q.	RENATA QUINTANILHA DOS SANTOS	2912
DOS SANTOS, R.Q.	RENATA QUINTANILHA DOS SANTOS	2918
DOS SANTOS, R.Q.	RENATA QUINTANILHA DOS SANTOS	2948
DOS SANTOS, R.T.T.	RAFAEL TEIXEIRA TIBURCIO DOS SANTOS	2775
DOS SANTOS, R.T.T.	RAFAEL TEIXEIRA TIBURCIO DOS SANTOS	3125
DOS SANTOS, S.L.B.	SILVIA LETICIA BISPO DOS SANTOS	3366
DOS SANTOS, T.C.	THIAGO CONCEIÇÃO DOS SANTOS	3406
DOS SANTOS, T.M.A.F.	TÂMILLA MAYANE ALVES FIDELIS DOS SANTOS	2634
DOS SANTOS, T.M.A.F.	TÂMILLA MAYANE ALVES FIDELIS DOS SANTOS	2667
DOS SANTOS, T.P.	THAÍS PIROLA DOS SANTOS	2772
DOS SANTOS, T.P.	THAÍS PIROLA DOS SANTOS	2809
DOS SANTOS, T.P.	THAÍS PIROLA DOS SANTOS	2829
DOS SANTOS, T.P.	THAÍS PIROLA DOS SANTOS	2832
DOS SANTOS, T.P.	THAÍS PIROLA DOS SANTOS	3034
DOS SANTOS, Y.B.	YASMIN BARROS DOS SANTOS	2968
DOURADO, L.P.S.	LÍVIA PIMENTEL SANT'ANA DOURADO	3142
DOURADO, L.P.S.	LÍVIA PIMENTEL SANT'ANA DOURADO	3332
DOURADO, L.P.S.	LÍVIA PIMENTEL SANT'ANA DOURADO	3385
DOURADO, L.P.S.	LÍVIA PIMENTEL SANT'ANA DOURADO	3392
DRUMM, M.E.G.O.	MARIA EDUARDA GÜNTHER OLIVEIRA DRUMM	3117
DRUMOND, B.P.	BETÂNIA PAIVA DRUMOND	2644
DRUMOND, B.P.	BETÂNIA PAIVA DRUMOND	2652
DUAN, S.	SUSU DUAN	3413
DUARTE, A.J.D.S.	ALBERTO JOSE DA SILVA DUARTE	2776
DUARTE, A.L.D.S.	ANNA LAURA DE SOUZA DUARTE	2987
DUARTE, C.K.	CAMILA KUMMEL DUARTE	2683
DUARTE, D.	DIEGO DUARTE	2951
DUARTE, F.O.	FERNANDA OLIVEIRA DUARTE	3255
DUARTE, F.O.	FERNANDA OLIVEIRA DUARTE	3297
DUARTE, M.S.V.	MARIANA SALDANHA VIEGAS DUARTE	3392
DUARTE-BARBOSA, M.	MÁRCIA DUARTE-BARBOSA	3042
DUENHA, E.D.S.	ESTER DA SILVA DUENHA	3286
DUGGAL, N.	NIHARIKA DUGGAL	2866
DUMOULIN, B.	BERNHARD DUMOULIN	3506
DURAO, C.V.	CESAR VIEIRA DURAO	2813
DURAO, C.V.	CESAR VIEIRA DURAO	2822
DURAO, C.V.	CESAR VIEIRA DURAO	2874
DURAO, C.V.	CESAR VIEIRA DURAO	2911
DURAO, C.V.	CESAR VIEIRA DURAO	3116
DURIGON, E.	EDISON DURIGON	2672
DURIGON, E.	EDISON DURIGON	2740
DURIGON, E.	EDISON DURIGON	2865
DURIGON, E.	EDISON DURIGON	3149
DUROCHER, B.	BARBARA DUROCHER	2901
DURSO, D.F.	DANIELLE FERNANDES DURSO	2643
DURSO, D.F.	DANIELLE FERNANDES DURSO	2645
DURUM, S.K.	SCOTT K. DURUM	2751
DUTRA, F.F.	FABIANNO FERREIRA DUTRA	2794
DUTRA, F.F.	FABIANNO FERREIRA DUTRA	2796
DUTRA, F.F.	FABIANNO FERREIRA DUTRA	2815
DUTRA, F.S.P.	FILIPE SANTOS PEREIRA DUTRA	3257
DUTRA, F.S.P.	FILIPE SANTOS PEREIRA DUTRA	3429

DUTRA, J.D.C.F.	JOYCE DA CRUZ FERRAZ DUTRA	2679
DUTRA, L.S.	LUANA SANTANA DUTRA	3393
DUTRA, S.	SIMONE DUTRA	3455
DUTRA, W.	WALDEREZ DUTRA	2790
DUTRA, W.	WALDEREZ DUTRA	2972
DUTRA, W.	WALDEREZ DUTRA	2976
DUTRA, W.	WALDEREZ DUTRA	3026
DUTRA, W.	WALDEREZ DUTRA	3028
DUTRA, W.	WALDEREZ DUTRA	3234
DUTRA, W.	WALDEREZ DUTRA	3293
EBERLIN, L.S.	LÍVIA SCHIAVINATO EBERLIN	3133
EBRAHIMABADI, S.	SIMA EBRAHIMABADI	2802
EBURNEO, G.S.	GABRIELA STRAFOLINO EBURNEO	2899
ELEUTÉRIO, B.P.	BRUNO PRADO ELEUTÉRIO	3124
ELEUTÉRIO, B.P.	BRUNO PRADO ELEUTÉRIO	3139
ELEUTÉRIO, B.P.	BRUNO PRADO ELEUTÉRIO	3223
ELIAS, B.D.D.S.	BRUNO DALBELO DA SILVA ELIAS	2827
ELIAS, B.D.D.S.	BRUNO DALBELO DA SILVA ELIAS	2841
ELIAS, B.D.D.S.	BRUNO DALBELO DA SILVA ELIAS	3094
ELIAS, B.D.D.S.	BRUNO DALBELO DA SILVA ELIAS	3171
ELIAS, B.D.D.S.	BRUNO DALBELO DA SILVA ELIAS	3309
ELIAS, L.L.K.	LUCILA LEICO KAGOHARA ELIAS	3269
EL-KADI, N.T.	NADIA TAVARES EL-KADI	3245
EMERICK, G.L.	GUILHERME LUZ EMERICK	2737
EMERICK, G.L.	GUILHERME LUZ EMERICK	3092
EMERICK, G.L.	GUILHERME LUZ EMERICK	3322
EMMERICK, K.R.	KAREN ROSALINO EMMERICK	3390
ESCOBAR, P.C.	PATRÍCIA CUERVO ESCOBAR	3009
ESCOLANO, A.	AMELIA ESCOLANO	3413
ESCOTE-FERRAZ, A.C.	ANA CAROLINA ESCOTE-FERRAZ	3277
ESLABÃO, L.B.	LÍVIA BUDZIAREK ESLABÃO	3449
ESPINDOLA, A.D.S.	ALINE DE SOUZA ESPINDOLA	3471
ESTEVES, K.S.	KAROLINA SANTOS ESTEVES	2861
ESTEVES, K.S.	KAROLINA SANTOS ESTEVES	2870
ESTEVES, K.S.	KAROLINA SANTOS ESTEVES	2959
ESTEVES, K.S.	KAROLINA SANTOS ESTEVES	2969
ESTEVES, K.S.	KAROLINA SANTOS ESTEVES	3016
ESTEVES, S.N.	SÉRGIO NOVITA ESTEVES	2782
EULÁLIO, M.D.M.C.	MICAELA DE MELO CORDEIRO EULÁLIO	2971
EULÁLIO, M.D.M.C.	MICAELA DE MELO CORDEIRO EULÁLIO	2983
EVANGELISTA, G.C.M.	GABRIELA COELI MENEZES EVANGELISTA	2855
EVANGELISTA, G.F.D.B.	GUILHERME FERREIRA DE BRITO EVANGELISTA	3121
EVANGELISTA, G.F.D.B.	GUILHERME FERREIRA DE BRITO EVANGELISTA	3134
EVANGELISTA, G.F.D.B.	GUILHERME FERREIRA DE BRITTO EVANGELISTA	2842
EVANGELISTA, G.F.D.B.	GUILHERME FERREIRA DE BRITTO EVANGELISTA	3317
EVANS, V.	VICTORIA EVANS	3487
EVAVOLD, C.	CHARLES EVAVOLD	3027
EYNDE, B.V.D.	BENOIT VAN DEN EYNDE	2929
FABRI, R.L.	RODRIGO LUIZ FABRI	3081
FABRI, R.L.	RODRIGO LUIZ FABRI	3162
FABRI, R.L.	RODRIGO LUIZ FABRI	3213
FABRI, R.L.	RODRIGO LUIZ FABRI	3458
FABRO, A.T.	ALEXANDRE T. FABRO	2970
FABRO, A.T.	ALEXANDRE TODOROVIC FABRO	3130
FABRO, A.T.	ALEXANDRE TODOROVIC FABRO	3185
FABRO, A.T.	ALEXANDRE TODOROVIC FABRO	3465
FACCIOLI, L.H.	LUCIA HELENA FACCIOLI	3344
FACCION, R.S.	ROBERTA SOARES FACCION	3174
FACCION, R.S.	ROBERTA SOARES FACCION	3184

FACCION, R.S.	ROBERTA SOARES FACCION	3187
FACCION, R.S.	ROBERTA SOARES FACCION	3249
FACCION, R.S.	ROBERTA SOARES FACCION	3268
FACCION, R.S.	ROBERTA SOARES FACCION	3371
FACCION, R.S.	ROBERTA SOARES FACCION	3411
FACHI, J.	JOSÉ FACHI	3348
FACHI, J.L.	JOSÉ LUÍS FACHI	2798
FACHI, J.L.	JOSÉ LUÍS FACHI	2843
FACURY, T.M.	TIAGO MOREIRA FACURY	3100
FAGUNDES, C.T.	CAIO TAVARES FAGUNDES	2657
FAIRLEY, J.K.	JESSICA K FAIRLEY	3219
FALCÃO, C.A.B.	CAMILA ALVES BANDEIRA FALCÃO	3399
FALLA, M.V.A.	MONICA VIVIANA ABREU FALLA	3326
FALQUETO, A.	ALOISIO FALQUETO	3123
FALQUETO, A.	ALOÍSIO FALQUETO	2862
FALQUETO, A.	ALOÍSIO FALQUETO	2868
FALQUETO, A.	ALOÍSIO FALQUETO	3403
FALQUETO, A.	ALOÍSIO FALQUETO	3483
FAMILIAR-MACEDO, D.	DÉBORA FAMILIAR-MACEDO	2742
FANG, Z.	ZHENHAO FANG	2719
FANTACINI, D.M.C.	DAIANNE MACIELY CARVALHO FANTACINI	3016
FANTIN, C.	CLEITON FANTIN	3323
FANTIN, J.	JULIANA FANTIN	2937
FANTIN, J.	JULIANA FANTIN	3474
FARDIN, J.M.	JULIA MIRANDA FARDIN	3059
FARDIN, J.M.	JULIA MIRANDA FARDIN	3227
FARDIN, J.M.	JULIA MIRANDA FARDIN	3403
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2643
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2645
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2656
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2664
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2665
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2673
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2676
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2677
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2678
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2679
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2682
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	2684
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	3059
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	3067
FARIA, A.M.C.	ANA MARIA CAETANO FARIA	3250
FARIA, J.A.Q.A.	JERUSA ARAUJO QUINTAO ARANTES FARIA	3414
FARIA, J.A.Q.A.	JERUSA ARAÚJO QUINTÃO ARANTES FARIA	2900
FARIA, J.M.D.L.	JOÃO MARCOS DE LIMA FARIA	2733
FARIAS, A.D.S.	ALESSANDRO DOS SANTOS FARIAS	2958
FARIAS, A.D.S.	ALESSANDRO DOS SANTOS FARIAS	2978
FARIAS, B.J.C.	BRAZ JUNIOR CAMPOS FARIAS	2971
FARIAS, G.R.	GABRIEL R. FARIAS	2763
FARIAS, L.P.	LEONARDO PAIVA FARIAS	3156
FARIAS, R.A.C.	RUTH AYLLA CALDEIRA FARIAS	2727
FAUSTINO, L.P.	LÍDIA PAULA FAUSTINO	2672
FAUSTINO, L.P.	LÍDIA PAULA FAUSTINO	2865
FAVORETTO, B.C.	BRUNA CRISTINA FAVORETTO	2894
FEITOSA, D.	DANIEL FEITOSA	3135
FEITOSA, M.R.	MARLEY RIBEIRO FEITOSA	3381
FEITOZA, J.M.S.	JOÃO MATEUS SILVA FEITOZA	2751
FEITOZA, J.M.S.	JOÃO MATEUS SILVA FEITOZA	2947
FEITOZA, J.M.S.	JOÃO MATEUS SILVA FEITOZA	2949

FEITOZA, J.M.S.	JOÃO MATEUS SILVA FEITOZA	2973
FELICE, F.	FERNANDA FELICE	2810
FELICE, F.	FERNANDA FELICE	3006
FELÍCIO, R.D.F.M.	RAFAELA DE FREITAS MARTINS FELÍCIO	3285
FELIPE, M.G.	MATHEUS GENNARI FELIPE	2954
FÉLIX, M.E.M.	MARIA EDUARDA MARTUCHELE FÉLIX	2670
FÉLIX, M.E.M.	MARIA EDUARDA MARTUCHELE FÉLIX	2687
FÉLIX, M.E.M.	MARIA EDUARDA MARTUCHELE FÉLIX	2690
FENILLI, G.P.	GABRIELA PIOVESAN FENILLI	3118
FERES, O.	OMAR FERES	3160
FERES, O.	OMAR FERES	3168
FERES, O.	OMAR FERES	3206
FERES, O.	OMAR FERES	3381
FERNANDES, A.M.S.	ANTÔNIO MÁRCIO SANTANA FERNANDES	3366
FERNANDES, A.M.S.	ANTÔNIO MÁRCIO SANTANA FERNANDES	3373
FERNANDES, A.M.S.	ANTÔNIO MÁRCIO SANTANA FERNANDES	3406
FERNANDES, A.M.S.	ANTÔNIO MÁRCIO SANTANA FERNANDES	3441
FERNANDES, A.M.S.	ANTÔNIO MÁRCIO SANTANA FERNANDES	3504
FERNANDES, A.P.	ANA PAULA FERNANDES	2641
FERNANDES, A.P.	ANA PAULA FERNANDES	2655
FERNANDES, A.P.	ANA PAULA FERNANDES	2661
FERNANDES, A.P.	ANA PAULA FERNANDES	2663
FERNANDES, A.P.	ANA PAULA FERNANDES	2669
FERNANDES, A.P.	ANA PAULA FERNANDES	3276
FERNANDES, A.R.	ALEXANDRE RIBEIRO FERNANDES	3184
FERNANDES, A.T.G.	ANA TERESA GOMES FERNANDES	3058
FERNANDES, B.V.R.	BARBARA VITORIA RODRIGUES FERNANDES	2933
FERNANDES, C.F.C.	CARLA FREIRE CELEDONIO FERNANDES	3514
FERNANDES, G.D.R.	GABRIEL DA ROCHA FERNANDES	3294
FERNANDES, G.D.R.	GABRIEL DA ROCHA FERNANDES	3395
FERNANDES, G.M.	GABRIELA MAIA FERNANDES	2666
FERNANDES, H.D.D.P.	HEINY DELCIENE DE PINA FERNANDES	2963
FERNANDES, J.	JANAINA FERNANDES	3109
FERNANDES, J.C.R.	JULIANE CRISTINA RIBEIRO FERNANDES	3157
FERNANDES, J.G.C.D.	JOÃO GABRIEL CAMPOS DOURADO FERNANDES	2690
FERNANDES, L.C.	LAUANE CASOLA FERNANDES	2755
FERNANDES, L.C.	LETÍCIA COUTO FERNANDES	2976
FERNANDES, L.G.R.	LUIS GUSTAVO ROMANI FERNANDES	2884
FERNANDES, L.G.R.	LUIS GUSTAVO ROMANI FERNANDES	3056
FERNANDES, L.M.	LUIZA MARIA FERNANDES	3189
FERNANDES, M.D.A.	MARIANA DE ANDRADE FERNANDES	2646
FERNANDES, M.K.C.	MAYARA KAREN CARVALHO FERNANDES	3248
FERNANDES, M.K.C.	MAYARA KAREN CARVALHO FERNANDES	3280
FERNANDES, M.K.C.	MAYARA KAREN CARVALHO FERNANDES	3302
FERNANDES, M.K.C.	MAYARA KAREN CARVALHO FERNANDES	3340
FERNANDES, M.R.	MARCELA ROMANAZZI FERNANDES	3163
FERNANDES, M.R.	MARCELA ROMANAZZI FERNANDES	3201
FERNANDES, N.A.R.	NATALIE APARECIDA RODRIGUES FERNANDES	3301
FERNANDES, P.D.	PATRICIA DIAS FERNANDES	3251
FERNANDES, R.	RICARDO FERNANDES	2929
FERNANDES, R.	RÚBIA FERNANDES	2676
FERNANDES, R.S.	RENATA SALGADO FERNANDES	2651
FERNANDES, R.S.	RENATA SALGADO FERNANDES	2672
FERNANDES-SANTOS, C.	CAROLINE FERNANDES-SANTOS	3052
FERNANDES-SANTOS, C.	CAROLINE FERNANDES-SANTOS	3057
FERNANDEZ, C.N.	CAMILA NASCIMENTO FERNANDEZ	3349
FERNANDEZ, C.N.	CAMILA NASCIMENTO FERNANDEZ	3507
FERRAIUOLO, H.C.C.	HENRIQUE CESAR CALDERON FERRAIUOLO	2809
FERRARI, M.D.F.R.	MERARI DE FÁTIMA RAMIRES FERRARI	3155

FERRARI, M.D.F.R.	MERARI DE FÁTIMA RAMIRES FERRARI	3290
FERRARINI, S.R.	STELA REGINA FERRARINI	2737
FERREIRA JR, O.C.	ORLANDO C. FERREIRA JR	2941
FERREIRA JUNIOR, R.S.	RUI SEABRA FERREIRA JUNIOR	3202
FERREIRA, A.A.F.E.	ALEX AUGUSTO FERREIRA E FERREIRA	2971
FERREIRA, A.A.F.E.	ALEX AUGUSTO FERREIRA E FERREIRA	2983
FERREIRA, A.C.A.	ANA CAROLINA ALMEIDA FERREIRA	3373
FERREIRA, A.C.A.	ANA CAROLINA ALMEIDA FERREIRA	3406
FERREIRA, A.C.A.	ANA CAROLINA ALMEIDA FERREIRA	3441
FERREIRA, A.C.A.	ANA CAROLINA ALMEIDA FERREIRA	3504
FERREIRA, A.M.	ANA MARÍA FERREIRA	2957
FERREIRA, A.P.	ANA PAULA FERREIRA	2847
FERREIRA, A.P.S.	ARYANNY PAULA SOUSA FERREIRA	2751
FERREIRA, A.P.S.	ARYANNY PAULA SOUSA FERREIRA	2949
FERREIRA, A.P.S.	ARYANNY PAULA SOUSA FERREIRA	2973
FERREIRA, A.V.M.	ADALIENE VERSIANE M. FERREIRA	3451
FERREIRA, B.R.	BEATRIZ ROSSETTI FERREIRA	2875
FERREIRA, B.R.	BEATRIZ ROSSETTI FERREIRA	3033
FERREIRA, B.R.	BEATRIZ ROSSETTI FERREIRA	3275
FERREIRA, C.E.D.S.	CARLOS EDUARDO DOS SANTOS FERREIRA	3133
FERREIRA, C.L.	CECILIO LEMES FERREIRA	3178
FERREIRA, C.M.G.	CAMILLY M. G. FERREIRA	2802
FERREIRA, D.C.	DANIELLE COZACHENCO FERREIRA	3166
FERREIRA, E.G.	ERICKA GUIMARAES FERREIRA	2885
FERREIRA, E.G.	ERICKA GUIMARAES FERREIRA	3328
FERREIRA, F.B.	FRANCIELLE BELINELLI FERREIRA	3270
FERREIRA, G.B.M.	GISELLE BENEVIDES MONTEIRO FERREIRA	3323
FERREIRA, G.M.	GEOVANE MARQUES FERREIRA	2670
FERREIRA, G.M.	GEOVANE MARQUES FERREIRA	2691
FERREIRA, G.M.	GEOVANE MARQUES FERREIRA	2692
FERREIRA, G.M.	GEOVANE MARQUES FERREIRA	2693
FERREIRA, G.M.	GEOVANE MARQUES FERREIRA	2714
FERREIRA, G.M.D.C.	GABRIELA MARIA DA COSTA FERREIRA	3188
FERREIRA, J.C.B.	JULIO CESAR BATISTA FERREIRA	3269
FERREIRA, J.P.E.	JOÃO PEDRO EMANUEL FERREIRA	3336
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	2897
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	2904
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	3099
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	3132
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	3138
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	3195
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	3238
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	3241
FERREIRA, J.R.M.	JESUINO RAFAEL MACHADO FERREIRA	3256
FERREIRA, L.C.D.L.	LUIZ CARLOS DE LIMA FERREIRA	3272
FERREIRA, L.C.D.S.	LUIS CARLOS DE SOUZA FERREIRA	2793
FERREIRA, L.D.S.	LUANA DA SILVA FERREIRA	3086
FERREIRA, L.D.S.	LUANA DA SILVA FERREIRA	3166
FERREIRA, L.D.S.	LUANA DA SILVA FERREIRA	3169
FERREIRA, L.M.	LETÍCIA MARTIMIANO FERREIRA	2888
FERREIRA, L.M.	LETÍCIA MARTIMIANO FERREIRA	3086
FERREIRA, L.M.A.	LÍVIA MARIA ALVES FERREIRA	2657
FERREIRA, L.V.L.	LEONARDO VILLA LEÃO FERREIRA	2927
FERREIRA, M.D.	MARCELA DAVOLI FERREIRA	2926
FERREIRA, M.F.	MIRIAM FRANCISCA FERREIRA	3439
FERREIRA, N.S.	NATALIA SOARES FERREIRA	3007
FERREIRA, R.	RAQUEL FERREIRA	2641
FERREIRA, R.A.	ROBLEDO AIRES FERREIRA	3472
FERREIRA, R.R.	ROBERTO RODRIGUES FERREIRA	3359

FERREIRA, R.R.	ROBERTO RODRIGUES FERREIRA	3404
FERREIRA, S.C.	SOPHIA COELHO FERREIRA	3083
FERREIRA, S.C.	SOPHIA COELHO FERREIRA	3272
FERREIRA, S.D.C.	SANDRO DA COSTA FERREIRA	3160
FERREIRA, S.D.C.	SANDRO DA COSTA FERREIRA	3168
FERREIRA, S.D.C.	SANDRO DA COSTA FERREIRA	3206
FERREIRA, S.M.I.	STEPHANIE MARTINEZ IASBEKE FERREIRA	2842
FERREIRA, S.M.I.	STEPHANIE MARTINEZ IASBEKE FERREIRA	3121
FERREIRA, S.M.I.	STEPHANIE MARTINEZ IASBEKE FERREIRA	3134
FERREIRA, T.D.S.	THAINA DA SILVA FERREIRA	3013
FERREIRA, T.D.S.	THAINA DA SILVA FERREIRA	3299
FERREIRA, T.D.S.	THAINA DA SILVA FERREIRA	3301
FERREIRA, T.R.	THAÍS ROMANO FERREIRA	3121
FERREIRA, T.R.	THAÍS ROMANO FERREIRA	3134
FERREIRA, T.R.	THAYNÁ RUIZ FERREIRA	2952
FERREIRA, T.R.	THAYNÁ RUIZ FERREIRA	3382
FERREIRA, W.S.	WELLINGTON SILVA FERREIRA	3304
FERROCO, C.L.D.V.	CLARA LUCY DE VASCONCELLOS FERROCO	2649
FERRONATTO, J.A.	JOSÉ AUGUSTO FERRONATTO	3010
FIALHO, S.N.	SAARA NERY FIALHO	3514
FIDALGO, G.	GABRIEL FIDALGO	2901
FIDALGO, T.M.	THIAGO M. FIDALGO	3423
FIELDING, C.A.	CERI ALAN FIELDING	2978
FIGUEIREDO, A.	AGNES FIGUEIREDO	2814
FIGUEIREDO, A.B.	AMANDA BRAGA FIGUEIREDO	2777
FIGUEIREDO, A.B.	AMANDA BRAGA FIGUEIREDO	2842
FIGUEIREDO, A.B.	AMANDA BRAGA FIGUEIREDO	2976
FIGUEIREDO, A.B.	AMANDA BRAGA FIGUEIREDO	3060
FIGUEIREDO, A.B.	AMANDA BRAGA FIGUEIREDO	3121
FIGUEIREDO, A.B.	AMANDA BRAGA FIGUEIREDO	3134
FIGUEIREDO, A.B.	AMANDA BRAGA FIGUEIREDO	3293
FIGUEIREDO, A.B.	AMANDA BRAGA FIGUEIREDO	3317
FIGUEIREDO, A.C.C.	ANNA CAROLINA CANÇADO FIGUEIREDO	2630
FIGUEIREDO, C.	CAMILA FIGUEIREDO	3312
FIGUEIREDO, E.P.	ELIANE PEREIRA FIGUEIREDO	3307
FIGUEIREDO, L.M.	LEANDER MATHIAS FIGUEIREDO	2891
FIGUEIREDO, M.M.	MARIA MARTA FIGUEIREDO	2636
FIGUEIREDO, M.M.	MARIA MARTA FIGUEIREDO	2639
FIGUEIREDO, M.M.	MARIA MARTA FIGUEIREDO	2658
FIGUEIREDO, M.M.	MARIA MARTA FIGUEIREDO	2672
FIGUEIREDO, M.M.	MARIA MARTA FIGUEIREDO	2865
FIGUEIREDO, R.T.	RODRIGO TINOCO FIGUEIREDO	3072
FIGUEIREDO, R.T.	RODRIGO TINOCO FIGUEIREDO	3090
FIGUEIREDO, V.	VILMA FIGUEIREDO	3065
FIGUEIREDO, V.G.	VIVIAN GONZALEZ FIGUEIREDO	2987
FIGUEIREDO, V.G.	VIVIAN GONZALEZ FIGUEIREDO	3165
FIGUEIREDO-VANZAN, D.	DANIEL FIGUEIREDO-VANZAN	3070
FILARDI, E.T.M.	ELOISE TROSTDORF MONTEIRO FILARDI	3163
FILARDI, E.T.M.	ELOISE TROSTDORF MONTEIRO FILARDI	3201
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	2897
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	2904
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3099
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3132
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3138
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3195
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3232
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3238
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3241
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3256

FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3261
FILARDY, A.D.	ALESSANDRA D'ALMEIDA FILARDY	3306
FILGUEIRAS, I.S.	IGOR SALERNO FILGUEIRAS	2854
FILGUEIRAS, I.S.	IGOR SALERNO FILGUEIRAS	3077
FILGUEIRAS, P.S.	PRISCILLA SOARES FILGUEIRAS	2756
FILGUEIRAS, P.S.	PRISCILLA SOARES FILGUEIRAS	3267
FILGUEIRAS, P.S.	PRISCILLA SOARES FILGUEIRAS	3294
FILIPPI, R.Z.	RENÉE ZON FILIPPI	3060
FINTELMAN-RODRIGUES, N.	NATALIA FINTELMAN-RODRIGUES	3479
IORESE, M.S.	MARCELA SENE IORESE	3255
FIORINI, A.C.	ALEX CARVALHO FIORINI	2641
FIORINI, A.C.	ALEX CARVALHO FIORINI	3276
FIRMINO, L.D.S.L.	LARYSSA DA SILVA LUZ FIRMINO	3469
FISCH, A.	ANDRESSA FISCH	2875
FISCH, A.	ANDRESSA FISCH	3033
FLATOW, E.A.	ELIZABETH ALEXANDRA FLATOW	3093
FLATOW, E.A.	ELIZABETH ALEXANDRA FLATOW	3101
FLATOW, E.A.	ELIZABETH ALEXANDRA FLATOW	3140
FLINTZ, R.	ROSANA FLINTZ	2742
FLÓ, M.	MARTÍN FLÓ	2957
FLOREY, O.	OLIVER FLOREY	2808
FLORIANO, G.C.	GABRIELA COELHO FLORIANO	3330
FLORSHEIM, E.B.	ESTHER BORGES FLORSHEIM	3315
FOGOLIN, C.S.	CARLA SANZOCHI FOGOLIN	2854
FOGOLIN, C.S.	CARLA SANZOCHI FOGOLIN	2855
FOGOLIN, C.S.	CARLA SANZOCHI FOGOLIN	3221
FOGUEL, D.	DEBORA FOGUEL	3278
FOLLE, M.	MAITE FOLLE	2957
FONSECA, C.T.	CRISTINA TOSCANO FONSECA	2646
FONSECA, C.T.	CRISTINA TOSCANO FONSECA	2660
FONSECA, J.R.F.	JOEY RAMONE FERREIRA FONSECA	2751
FONSECA, J.R.F.	JOEY RAMONE FERREIRA FONSECA	2949
FONSECA, J.R.F.	JOEY RAMONE FERREIRA FONSECA	2973
FONSECA, M.J.P.	MARCIO J. P. FONSECA	2763
FONSECA, R.J.C.	ROBERTO JOSÉ CASTRO FONSECA	3467
FONTANARI, C.	CAROLINE FONTANARI	2867
FONTANARI, C.	CAROLINE FONTANARI	3018
FONTÃO, A.P.A.	ANA PAULA ALVES FONTÃO	2901
FONTES, W.	WAGNER FONTES	2839
FORMIGA JUNIOR, M.A.	MARCOS A. FORMIGA JUNIOR	2907
FORMIGA JUNIOR, M.A.	MARCOS A. FORMIGA JUNIOR	2999
FORMIGA JUNIOR, M.A.	MARCOS A. FORMIGA JUNIOR	3009
FORTUNATO, C.E.S.	CELSE EDUARDO SILVA FORTUNATO	2695
FORTUNATO, C.E.S.	CELSE EDUARDO SILVA FORTUNATO	2696
FORTUNATO, C.E.S.	CELSE EDUARDO SILVA FORTUNATO	2697
FORTUNATO, C.E.S.	CELSE EDUARDO SILVA FORTUNATO	2700
FORTUNATO, C.E.S.	CELSE EDUARDO SILVA FORTUNATO	2703
FORTUNATO, C.E.S.	CELSE EDUARDO SILVA FORTUNATO	2704
FRACAZZO, M.C.C.	MARIA CATARINA CAVALCANTI FRACAZZO	2867
FRACAZZO, M.C.C.	MARIA CATARINA CAVALCANTI FRACAZZO	3018
FRADE, M.A.C.	MARCO ANDREY CIPRIANI FRADE	3401
FRADICO, J.R.B.	JORDANA RODRIGUES BARBOSA FRADICO	2635
FRADICO, J.R.B.	JORDANA RODRIGUES BARBOSA FRADICO	2644
FRADICO, J.R.B.	JORDANA RODRIGUES BARBOSA FRADICO	2652
FRAGA JUNIOR, V.D.S.	VANDERLEI DA SILVA FRAGA JUNIOR	2728
FRAGA JUNIOR, V.D.S.	VANDERLEI DA SILVA FRAGA JUNIOR	3422
FRAGA JUNIOR, V.D.S.	VANDERLEI DA SILVA FRAGA JUNIOR	3473
FRAGA JUNIOR, V.D.S.	VANDERLEI DA SILVA FRAGA JUNIOR	3478
FRAGA, D.B.M.	DEBORAH BITTENCOURT MOTHE FRAGA	2775

FRAGA, D.B.M.	DEBORAH BITTENCOURT MOTHE FRAGA	3291
FRAGA, L.A.D.O.	LUCIA ALVES DE OLIVEIRA FRAGA	3219
FRAGELLI, B.D.D.L.	BRUNA DIAS DE LIMA FRAGELLI	2819
FRAGELLI, B.D.D.L.	BRUNA DIAS DE LIMA FRAGELLI	3255
FRAGELLI, B.D.D.L.	BRUNA DIAS DE LIMA FRAGELLI	3297
FRANÇA, A.S.	AMANDA SOARES FRANÇA	3307
FRANÇA, C.G.M.	CARLOS GABRIEL MORAIS FRANÇA	3398
FRANÇA, F.B.F.	FLÁVIA BATISTA FERREIRA FRANÇA	2689
FRANÇA, F.L.D.S.	FERNANDA LEMBO DE SOUZA FRANÇA	2874
FRANÇA, F.L.D.S.	FERNANDA LEMBO DE SOUZA FRANÇA	2911
FRANÇA, L.V.	LUÍSA VALÉRIO FRANCA	3127
FRANÇA, P.R.D.C.	PATRÍCIA RIBEIRO DE CARVALHO FRANÇA	3251
FRANÇA, R.F.D.O.	RAFAEL FREITAS DE OLIVEIRA FRANÇA	3369
FRANÇA, R.K.A.D.O.	RENATO KAYLAN ALVES DE OLIVEIRA FRANÇA	3103
FRANÇA, R.K.A.D.O.	RENATO KAYLAN ALVES DE OLIVEIRA FRANÇA	3190
FRANÇA, T.D.S.	TALITA DA SILVA FRANÇA	2778
FRANCO, F.N.	FILIPE NOGUEIRA FRANCO	3515
FRANCO, G.D.O.	GABRIELA DE OLIVEIRA FRANCO	2985
FRANCO, G.D.O.	GABRIELA DE OLIVEIRA FRANCO	3222
FRANTZ, F.G.	FABIANI G. FRANTZ	2867
FRANTZ, F.G.	FABIANI G. FRANTZ	3018
FREDERICO, A.B.T.	ANA BEATRIZ TEIXEIRA FREDERICO	3154
FREIRE, A.S.	AMANDA SANTOS FREIRE	2759
FREIRE, L.L.	LUCAS L. FREIRE	2927
FREIRE, M.	MONIQUE FREIRE	3272
FREIRE-ANTUNES, L.	LUCAS FREIRE-ANTUNES	2786
FREIRE-ANTUNES, L.	LUCAS FREIRE-ANTUNES	2849
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	2738
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	2862
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	2885
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	2912
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	2936
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	2948
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	3232
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	3403
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	3418
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	3427
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	3463
FREIRE-DE-LIMA, C.G.	CÉLIO GERALDO FREIRE-DE-LIMA	3484
FREITAS FILHO, E.G.	EDISMAURO GARCIA FREITAS FILHO	2808
FREITAS FILHO, E.G.	EDISMAURO GARCIA FREITAS FILHO	3084
FREITAS, B.A.D.A.	BETHÂNIA ALVES DE AVELAR FREITAS	2709
FREITAS, B.A.D.A.	BETHÂNIA ALVES DE AVELAR FREITAS	2710
FREITAS, B.A.D.A.	BETHÂNIA ALVES DE AVELAR FREITAS	2718
FREITAS, D.	DEISE FREITAS	3353
FREITAS, D.A.	DANIEL ALMEIDA FREITAS	2710
FREITAS, D.G.A.	DEBORA GOMES ALBUQUERQUE FREITAS	3077
FREITAS, H.C.	HELANO CARIOCA FREITAS	3121
FREITAS, H.C.	HELANO CARIOCA FREITAS	3134
FREITAS, R.	RODRIGO FREITAS	2989
FREITAS, R.	RODRIGO FREITAS	3008
FREITAS, R.	RODRIGO FREITAS	3230
FREITAS, R.J.R.X.	RODRIGO JOSE ROCHA XAVIER FREITAS	3325
FRETES, M.	MATHEUS FRETES	2880
FRIES, F.	FERNANDA FRIES	3455
FRIZZO, I.B.	IZADORA BORGMANN FRIZZO	3449
FRONY, A.C.	ANA CLARA FRONY	2925
FROZZA, F.T.B.	FERNANDA TEREZA BOVI FROZZA	3031
FROZZA, F.T.B.	FERNANDA TEREZA BOVI FROZZA	3289

FROZZA, F.T.B.	FERNANDA TEREZA BOVI FROZZA	3353
FROZZA, F.T.B.	FERNANDA TEREZA BOVI FROZZA	3488
FROZZA, R.L.	RUDIMAR LUIZ FROZZA	2876
FROZZA, R.L.	RUDIMAR LUIZ FROZZA	2931
FUENTES, D.L.P.	DEIVYS LEANDRO PORTUONDO FUENTES	3080
FUJIMOTO, V.C.M.	VICTÓRIA CRISTINA MENDONÇA FUJIMOTO	3445
FUMAGALLI, M.J.	MARCILIO JORGE FUMAGALLI	3007
FURTADO, G.P.	GILVAN PESSOA FURTADO	3135
FURTADO, I.P.	IZADORA PETER FURTADO	2861
FURTADO, I.P.	IZADORA PETER FURTADO	2870
FURTADO, I.P.	IZADORA PETER FURTADO	2959
FURTADO, I.P.	IZADORA PETER FURTADO	2969
FURTADO, I.P.	IZADORA PETER FURTADO	3016
GABRIELLE, B.	BÁRBARA GABRIELLE	2818
GABRIELLE, B.	BÁRBARA GABRIELLE	2941
GABRIELLE, B.	BÁRBARA GABRIELLE	2977
GABRILI, J.J.M.	JOEL JOSÉ MEGALE GABRILI	3319
GAITÁN, X.A.	XIOMARA ALEXANDRA GAITÁN	2950
GALANO, J.D.	JULIA DOMENE GALANO	3038
GALANTE, P.A.F.	PEDRO ALEXANDRE FAVORETTO GALANTE	3126
GALDINO, G.	GABRIELLE GALDINO	3505
GALDINO, M.E.F.	MARIA EDUARDA FERNANDES GALDINO	3014
GALDINO, N.A.D.L.	NAYANE ALVES DE LIMA GALDINO	3317
GALLARDO, Z.M.C.	ZOÉ MAGALÍ CEJAS GALLARDO	2840
GALLIEZ, R.	RAFAEL GALLIEZ	2941
GALLIEZ, R.	RAFAEL GALLIEZ	2977
GALON, J.	JÉRÔME GALON	3077
GALVANI, R.G.A.	RÔMULO GONÇALVES AGOSTINHO GALVANI	3502
GALVAO, I.	IZABELA GALVAO	2701
GALVÃO, L.	LUCIA GALVÃO	2972
GAMA, A.L.P.R.	ALBA LUCÍNIA PEIXOTO RANGEL GAMA	3377
GAMA, A.L.P.R.	ALBA LUCÍNIA PEIXOTO RANGEL GAMA	3405
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	2794
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	2815
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	2941
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	2963
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	2977
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	3086
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	3166
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	3169
GAMA, A.M.D.S.	ANDREZA MOREIRA DOS SANTOS GAMA	3253
GAMEIRO, J.	JACY GAMEIRO	3248
GAMEIRO, J.	JACY GAMEIRO	3280
GAMEIRO, J.	JACY GAMEIRO	3287
GAMEIRO, J.	JACY GAMEIRO	3302
GAMEIRO, J.	JACY GAMEIRO	3330
GAMEIRO, J.	JACY GAMEIRO	3331
GAMEIRO, J.	JACY GAMEIRO	3336
GAMEIRO, J.	JACY GAMEIRO	3340
GAMEIRO, J.	JACY GAMEIRO	3342
GAMEIRO, J.	JACY GAMEIRO	3458
GAMON, T.H.	THAIS HELENA GAMON	2865
GAN, F.C.	FLÁVIA CAROLINE GAN	3365
GANDINI, M.	MARIANA GANDINI	3065
GANDINI, M.	MARIANA GANDINI	3245
GANNAVARAM, S.	SREENIVAS GANNAVARAM	3210
GARCIA, A.B.	ALVARO BAEZA GARCIA	3050
GARCIA, A.B.	ANA BEATRIZ GARCIA	2994
GARCIA, B.C.C.	BRUNA CAROLINE CHAVES GARCIA	2987

GARCIA, B.C.C.	BRUNA CAROLINE CHAVES GARCIA	3165
GARCIA, C.	CHRISTIAN GARCIA	2804
GARCIA, D.D.A.	DIEGO DE ANDRADE GARCIA	2825
GARCIA, D.D.A.	DIEGO DE ANDRADE GARCIA	2830
GARCIA, D.D.A.	DIEGO DE ANDRADE GARCIA	2859
GARCIA, D.D.A.	DIEGO DE ANDRADE GARCIA	2864
GARCIA, D.D.A.	DIEGO DE ANDRADE GARCIA	2881
GARCIA, D.D.A.	DIEGO DE ANDRADE GARCIA	2888
GARCIA, D.D.A.	DIEGO DE ANDRADE GARCIA	2901
GARCÍA, H.D.M.	HENRY DAVID MOGOLLÓN GARCÍA	3111
GARCIA, M.A.D.N.	MIGUEL ANTÔNIO DO NASCIMENTO GARCIA	2779
GARCIA, M.E.M.	MARIA EDUARDA MONTEIRO GARCIA	3160
GARCIA, M.E.M.	MARIA EDUARDA MONTEIRO GARCIA	3168
GARCIA, M.E.M.	MARIA EDUARDA MONTEIRO GARCIA	3206
GARCIA, M.R.D.O.	MONIQUE RAMOS DE OLIVEIRA GARCIA	3329
GARCIA, V.F.D.R.	VITÓRIA FERNANDA DO ROSÁRIO GARCIA	3328
GARDINASSI, L.G.A.	LUIZ GUSTAVO ARAUJO GARDINASSI	2875
GARDINASSI, L.G.A.	LUIZ GUSTAVO ARAUJO GARDINASSI	3376
GARGANO, R.G.	RONALDO GOMES GARGANO	3010
GARRIDO, M.	MARCELO GARRIDO	2736
GASPAR, E.B.	EMANUELLE BALDO GASPAR	2906
GASPAR, E.B.	EMANUELLE BALDO GASPAR	3096
GASPAR, E.B.	EMANUELLE BALDO GASPAR	3450
GASPAR, R.C.	RAFAEL CALAIS GASPAR	2869
GASPAR, S.L.D.F.	SILVIA LETÍCIA DE FRANÇA GASPAR	3522
GASPAR, V.C.	VITÓRIA CHRISTINA GASPAR	2817
GASPARI, P.D.M.	PRISCYLA DANIELY MARCATO GASPARI	3357
GASSEN, R.B.	RODRIGO BENEDETTI GASSEN	2804
GASSEN, R.B.	RODRIGO BENEDETTI GASSEN	3031
GASSEN, R.B.	RODRIGO BENEDETTI GASSEN	3254
GASSEN, R.B.	RODRIGO BENEDETTI GASSEN	3353
GASSEN, R.B.	RODRIGO BENEDETTI GASSEN	3368
GASSEN, R.B.	RODRIGO BENEDETTI GASSEN	3488
GATTI, Í.D.M.V.	ÍTALA DE MORAES VIEIRA GATTI	2759
GAUDIO, R.	RODRIGO GAUDIO	2810
GAUDIO, R.	RODRIGO GAUDIO	3006
GAUDIO, R.	RODRIGO GAUDIO	3136
GAUI, M.D.F.D.	MARIA DE FATIMA DIAS GAUI	2901
GAVAZZONI, M.F.R.	MARIA FERNANDA REIS GAVAZZONI	3245
GAYER, C.R.M.	CARLOS ROBERTO MACHADO GAYER	3017
GAZUMYAN, A.	ANNA GAZUMYAN	3007
GAZZINELLI, I.H.	ISABELA HIRAKO GAZZINELLI	2658
GAZZINELLI, R.T.	RICARDO T. GAZZINELLI	2641
GAZZINELLI, R.T.	RICARDO T. GAZZINELLI	2650
GAZZINELLI, R.T.	RICARDO T. GAZZINELLI	2865
GAZZINELLI, R.T.	RICARDO T. GAZZINELLI	3276
GAZZINELLI, R.T.	RICARDO T. GAZZINELLI	3428
GAZZINELLI, R.T.	RICARDO T. GAZZINELLI	3509
GAZZINELLI, R.T.	RICARDO TOSTES GAZZINELLI	2636
GAZZINELLI, R.T.	RICARDO TOSTES GAZZINELLI	2639
GAZZINELLI, R.T.	RICARDO TOSTES GAZZINELLI	2651
GAZZINELLI, R.T.	RICARDO TOSTES GAZZINELLI	2658
GAZZINELLI, R.T.	RICARDO TOSTES GAZZINELLI	2663
GAZZINELLI, R.T.	RICARDO TOSTES GAZZINELLI	2672
GAZZINELLI, R.T.	RICARDO TOSTES GAZZINELLI	2715
GELTINK, R.K.	RAMON KLEIN GELTINK	2926
GEMBRE, A.F.	ANA FLÁVIA GEMBRE	3188
GENARO, L.M.	LÍVIA MOREIRA GENARO	3067
GENTIL, G.F.D.S.	GABRIELA FIGUEIRA DA SILVA GENTIL	3186

GERALDO, K.M.	KIM MATTOS GERALDO	3000
GERALDO, K.M.	KIM MATTOS GERALDO	3105
GERALDO, K.M.	KIM MATTOS GERALDO	3240
GERALDO, K.M.	KIM MATTOS GERALDO	3260
GESU, R.S.W.D.	REGINA S. W. DI GESU	3360
GHAFOURI, B.	BIJAR GHAFOURI	2932
GHOSN, E.	ELIVER GHOSN	2789
GIANNOTTI, B.B.	BRUNNA BARBOSA GIANNOTTI	2673
GIDLUND, M.	MAGNUS GIDLUND	3281
GIL, C.D.	CRISTIANE D. GIL	3143
GIL, H.G.	HELEN GAMA GIL	3257
GIL, H.G.	HELEN GAMA GIL	3439
GILFILLAN, S.	SUSAN GILFILLAN	2843
GIMENES, S.N.C.	SARAH NATALIE CIRILO GIMENES	3225
GIMENES, S.N.C.	SARAH NATALIE CIRILO GIMENES	3277
GIMENES, S.N.C.	SARAH NATHALIE CRIRILLO GIMENES	3218
GIRARDI, C.S.	CAROLINA SAIBRO GIRARDI	3503
GODARD, A.L.B.	ANA LUCIA BRUNIALTI GODARD	2676
GODARD, A.L.B.	ANA LUCIA BRUNIALTI GODARD	2684
GODINHO, I.P.	IGOR PEREIRA GODINHO	2674
GODOI, E.A.	ESTHER ALVES GODOI	2839
GOES, W.M.	WANEISSA M. GOES	3276
GOLDMAN, G.H.	GUSTAVO HENRIQUE GOLDMAN	2808
GOLDMAN, G.H.	GUSTAVO HENRIQUE GOLDMAN	3080
GOLDSTEIN, G.N.R.	GABRIEL NASSAR REICH GOLDSTEIN	2882
GOLLOB, K.J.	KENNETH JOHN GOLLOB	2777
GOLLOB, K.J.	KENNETH JOHN GOLLOB	2842
GOLLOB, K.J.	KENNETH JOHN GOLLOB	2972
GOLLOB, K.J.	KENNETH JOHN GOLLOB	2976
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3028
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3060
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3121
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3133
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3134
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3234
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3293
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3314
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3317
GOLLOB, K.J.	KENNETH JOHN GOLLOB	3364
GOMES, A.C.F.	ANA CAROLINA FIGUEIRINHA GOMES	2918
GOMES, A.I.N.	ANA IZABELE NUNES GOMES	3232
GOMES, A.I.N.	ANA IZABELE NUNES GOMES	3418
GOMES, C.B.D.S.	CRISTOPHER BRYAN DA SILVA GOMES	3347
GOMES, C.B.S.	CRISTOPHER B. S. GOMES	2632
GOMES, C.B.S.	CRISTOPHER B. S. GOMES	2633
GOMES, C.B.S.	CRISTOPHER B. S. GOMES	2648
GOMES, C.B.S.	CRISTOPHER B. S. GOMES	3509
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	2787
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	2862
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	2868
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	2898
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	3059
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	3123
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	3227
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	3409
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	3483
GOMES, D.C.D.O.	DANIEL CLAUDIO DE OLIVEIRA GOMES	3489
GOMES, D.L.R.	DÉBORA LEANDRO RAMA GOMES	3399
GOMES, E.M.	ELIANE MELLO GOMES	2734

GOMES, F.I.F.	FRANCISCO I F GOMES	3022
GOMES, F.I.F.	FRANCISCO I F GOMES	3054
GOMES, F.I.F.	FRANCISCO ISAAC FERNANDES GOMES	2852
GOMES, F.L.R.	FLÁVIA LIMA RIBEIRO GOMES	2786
GOMES, F.L.R.	FLÁVIA LIMA RIBEIRO GOMES	2834
GOMES, F.L.R.	FLÁVIA LIMA RIBEIRO GOMES	2849
GOMES, I.	ISABELA GOMES	3509
GOMES, I.P.	ISABELA PEREIRA GOMES	2641
GOMES, I.P.	ISABELA PEREIRA GOMES	3276
GOMES, L.G.	LEONARDO GONÇALVES GOMES	3449
GOMES, L.H.F.	LEONARDO HENRIQUE FERREIRA GOMES	3174
GOMES, L.R.	LETÍCIA RICCIO GOMES	2940
GOMES, M.F.D.C.	MARCELO FERREIRA DA COSTA GOMES	2756
GOMES, M.F.D.C.	MARCELO FERREIRA DA COSTA GOMES	3267
GOMES, M.T.	MILENA TAVARES GOMES	2834
GOMES, M.T.	MILENA TAVARES GOMES	3273
GOMES, M.T.	MILENA TAVARES GOMES	3456
GOMES, P.S.	POLLYANNA STEPHANIE GOMES	2838
GOMES, R.D.O.	RENATA DE OLIVEIRA GOMES	3370
GOMEZ, D.	DIANA GOMEZ	3276
GOMEZ, D.	DIANA GOMEZ	3509
GONÇALVES, A.	AMANDA GONÇALVES	3115
GONÇALVES, A.P.	ANDREZA PARREIRAS GONÇALVES	2642
GONÇALVES, A.P.	ANDREZA PARREIRAS GONÇALVES	2644
GONÇALVES, A.P.	ANDREZA PARREIRAS GONÇALVES	2652
GONÇALVES, B.D.S.	BRENNO DOS SANTOS GONÇALVES	3243
GONÇALVES, B.D.S.	BRENNO DOS SANTOS GONÇALVES	3249
GONÇALVES, B.Ô.P.	BRYAN ÔRTERO PEREZ GONÇALVES	2716
GONÇALVES, B.Ô.P.	BRYAN ÔRTERO PEREZ GONÇALVES	2717
GONÇALVES, B.S.	BARBARA SIMONSON GONÇALVES	2856
GONÇALVES, B.S.	BARBARA SIMONSON GONÇALVES	2872
GONÇALVES, B.S.	BARBARA SIMONSON GONÇALVES	2930
GONÇALVES, B.S.	BARBARA SIMONSON GONÇALVES	2999
GONÇALVES, B.S.	BARBARA SIMONSON GONÇALVES	3009
GONÇALVES, B.T.	BRUNA TIRAPELLI GONÇALVES	2827
GONÇALVES, H.C.	HILARY CESÁRIO GONÇALVES	3062
GONÇALVES, Í.D.S.	ÍTALO DA SILVA GONÇALVES	3250
GONÇALVES, Í.D.S.	ÍTALO DA SILVA GONÇALVES	3298
GONÇALVES, Í.D.S.	ÍTALO DA SILVA GONÇALVES	3464
GONÇALVES, J.V.P.	JOÃO VÍTOR PENDEZA GONÇALVES	3117
GONÇALVES, J.V.P.	JOÃO VÍTOR PENDEZA GONÇALVES	3254
GONÇALVES, J.V.P.	JOÃO VÍTOR PENDEZA GONÇALVES	3368
GONÇALVES, L.B.	LUKAS BOLINI GONÇALVES	3328
GONÇALVES, L.E.D.	LUÍS EDUARDO DUARTE GONÇALVES	2975
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2673
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2734
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2735
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2741
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2878
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2889
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2938
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2944
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2966
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2967
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	2982
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	3067
GONÇALVES, L.M.	LEONARDO MANDU GONÇALVES	3076
GONÇALVES, M.	MATHEUS GONÇALVES	3041
GONÇALVES, M.	MATHEUS GONÇALVES	3071

GONCALVES, M.D.S.	MARCELLE DA SILVA GONCALVES	3425
GONÇALVES, M.O.	MARIANA OTTAIANO GONÇALVES	2726
GONÇALVES, R.	RICARDO GONÇALVES	2671
GONÇALVES, R.	RICARDO GONÇALVES	2681
GONÇALVES, R.	RICARDO GONÇALVES	2688
GONCALVES, R.M.	REMY MARTINS GONCALVES	3049
GONCALVES, R.M.	REMY MARTINS GONCALVES	3329
GONCALVES, R.M.	REMY MARTINS GONCALVES	3479
GONCALVES, R.M.	REMY MARTINS GONCALVES	3490
GONÇALVES, T.A.R.P.	THALITA APARECIDA RIUL PRADO GONÇALVES	3129
GONGORA, A.	ALYSÉE GONGORA	3074
GONTIJO, V.S.	VANESSA SILVA GONTIJO	3251
GONZALEZ, C.S.	CAROL SERNA GONZALEZ	2954
GONZALEZ, G.	GUALBERTO GONZALEZ	2951
GOTO, H.	HIRO GOTO	3281
GOUDOURIS, E.S.	EKATERINI SIMÕES GOUDOURIS	3371
GOULART, G.A.C.	GISELE ASSIS CASTRO GOULART	2661
GOULART, J.P.	JAQUELINE PEGORETTI GOULART	3409
GOULART, M.N.G.	MILENA NEIRA GUIMARÃES GOULART	3000
GOULART, M.N.G.	MILENA NEIRA GUIMARÃES GOULART	3105
GOULART, M.N.G.	MILENA NEIRA GUIMARÃES GOULART	3240
GOULART, M.N.G.	MILENA NEIRA GUIMARÃES GOULART	3260
GOULART, M.T.	MELISSA TEIXEIRA GOULART	2937
GOULART, M.T.	MELISSA TEIXEIRA GOULART	3474
GOULART, P.P.G.	PEDRO PIRES GUIMARAES GOULART	2673
GOULART, P.P.G.	PEDRO PIRES GUIMARAES GOULART	2988
GOZZI, I.C.C.	ISABELLE CAROLINA COTRIM GOZZI	2761
GOZZI, I.C.C.	ISABELLE CAROLINA COTRIM GOZZI	2771
GOZZI, I.C.C.	ISABELLE CAROLINA COTRIM GOZZI	2791
GOZZI, I.C.C.	ISABELLE CAROLINA COTRIM GOZZI	2793
GRANADO, E.T.	EUGÊNIA TERRA GRANADO	3151
GRAZIANI, J.B.	JÚLIA BALARIN GRAZIANI	2777
GRAZIELLE, V.	VIVIANE GRAZIELLE	2655
GRIFONI, A.	ALBA GRIFONI	2719
GRIGORIOU, M.	MILENA GRIGORIOU	3155
GRINSZTEJN, B.	BEATRIZ GRINSZTEJN	3000
GRINSZTEJN, B.	BEATRIZ GRINSZTEJN	3064
GRINSZTEJN, B.	BEATRIZ GRINSZTEJN	3105
GRINSZTEJN, B.	BEATRIZ GRINSZTEJN	3240
GRINSZTEJN, B.	BEATRIZ GRINSZTEJN	3260
GRINSZTEJN, B.	BEATRIZ GRINSZTEJN	3362
GRIPP, C.B.W.G.	CARMEM BEATRIZ WAGNER GIACOIA GRIPP	3000
GRIPP, C.B.W.G.	CARMEM BEATRIZ WAGNER GIACOIA GRIPP	3105
GRIPP, C.B.W.G.	CARMEM BEATRIZ WAGNER GIACOIA GRIPP	3240
GRIPP, C.B.W.G.	CARMEM BEATRIZ WAGNER GIACOIA GRIPP	3260
GROSS, J.L.	JEFFERSON LUIZ GROSS	3121
GROSS, J.L.	JEFFERSON LUIZ GROSS	3134
GRUBAUGH, N.	NATHAN GRUBAUGH	2719
GRUPP, S.	STEPHAN GRUPP	3142
GUADAGNINI, D.	DIOZE GUADAGNINI	2882
GUALBERTO, A.	ANA GUALBERTO	3510
GUAMÁN-BURNEO, M.C.	MARÍA CRISTINA GUAMÁN-BURNEO	2714
GUANAES, J.O.F.	JÉSSICA OLIVEIRA FRADE GUANAES	3360
GUARDALINI, L.G.O.	LUIS GIOVANI OLIVEIRA GUARDALINI	3351
GUARNEIRE, G.J.	GRACIMÉRIO JOSÉ GUARNEIRE	3046
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	2823
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	2838
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	2862
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	2980

GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3164
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3232
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3343
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3346
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3349
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3363
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3403
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3484
GUEDES, H.L.D.M.	HERBERT LEONEL DE MATOS GUEDES	3507
GUEDES, I.A.	ISABELLA ALVIM GUEDES	3251
GUEDES, M.C.M.R.	MARIA CLARA MACHADO RESENDE GUEDES	3081
GUEDES, M.C.M.R.	MARIA CLARA MACHADO RESENDE GUEDES	3082
GUEDES, M.C.M.R.	MARIA CLARA MACHADO RESENDE GUEDES	3102
GUEDES, M.C.M.R.	MARIA CLARA MACHADO RESENDE GUEDES	3162
GUEDES, M.L.	MARIA LENISE GUEDES	3406
GUÉRIN, D.M.A.	DIEGO MARCELO A GUÉRIN	3453
GUERRA, F.S.	FELIPE SARMENTO GUERRA	3341
GUERRA, R.O.	RHANOICA OLIVEIRA GUERRA	3369
GUERRA, R.O.	RHANOICA OLIVEIRA GUERRA	3384
GUERREIRO, M.A.D.S.	MARIA ALICE DE SOUZA GUERREIRO	2825
GUERREIRO, M.A.D.S.	MARIA ALICE DE SOUZA GUERREIRO	2830
GUERREIRO, M.A.D.S.	MARIA ALICE DE SOUZA GUERREIRO	2859
GUERREIRO, M.A.D.S.	MARIA ALICE DE SOUZA GUERREIRO	2864
GUERREIRO, M.A.D.S.	MARIA ALICE DE SOUZA GUERREIRO	2881
GUIMARÃES, A.C.G.	ANA CLARA GAZZINELLI GUIMARÃES	2641
GUIMARÃES, E.S.	ERIKA SOUSA GUIMARÃES	2828
GUIMARÃES, E.T.	ELISALVA TEIXEIRA GUIMARÃES	3341
GUIMARÃES, F.	FERNANDO GUIMARÃES	2947
GUIMARÃES, F.S.	FRANCISCO SILVEIRA GUIMARÃES	3022
GUIMARÃES, F.V.	FERNANDA VERDINI GUIMARÃES	3278
GUIMARÃES, H.C.	HENRIQUE CERQUEIRA GUIMARÃES	2645
GUIMARÃES, J.M.C.S.	JOSÉ MÁRIO COSTA SANTANA GUIMARÃES	2921
GUIMARÃES, J.M.C.S.	JOSÉ MÁRIO COSTA SANTANA GUIMARÃES	3167
GUIMARÃES, J.M.C.S.	JOSÉ MÁRIO COSTA SANTANA GUIMARÃES	3209
GUIMARÃES, M.L.	MONICK LINDENMEYER GUIMARÃES	3064
GUIMARÃES, P.O.	PIETRA OLIVEIRA GUIMARÃES	3053
GUIMARÃES, P.O.	PIETRA OLIVEIRA GUIMARÃES	3389
GUIMARAES, P.P.G.	PEDRO PIRES GOULART GUIMARAES	2686
GUIMARAES, P.P.G.	PEDRO PIRES GOULART GUIMARAES	3032
GUIMARÃES, R.L.	RAISSA LUDWIG GUIMARÃES	2889
GUIMARÃES, R.M.	RAFAELA MANO GUIMARÃES	3022
GUIMARÃES, V.H.	VICTOR HUGO GUIMARÃES	3255
GULARTE, D.	DANIEL GULARTE	3135
GUNDERMAN, V.H.G.	VICTOR HUGO GONÇALVES GUNDERMAN	2636
GUNDERMAN, V.H.G.	VICTOR HUGO GONÇALVES GUNDERMAN	2639
GUSMAO, A.F.	ARIANNE FAGOTTI GUSMAO	2827
GUSMAO, A.F.	ARIANNE FAGOTTI GUSMAO	2841
GUSMAO, A.F.	ARIANNE FAGOTTI GUSMAO	2854
GUSMAO, A.F.	ARIANNE FAGOTTI GUSMAO	3029
GUSMAO, A.F.	ARIANNE FAGOTTI GUSMAO	3094
GUSMAO, A.F.	ARIANNE FAGOTTI GUSMAO	3171
GUSMAO, A.F.	ARIANNE FAGOTTI GUSMAO	3309
GUSTINELI, B.P.	BÁRBARA PASTORI GUSTINELI	3024
HAACH, V.	VANESSA HAACH	2780
HAGAN, T.	THOMAS HAGAN	3131
HALL, N.V.P.A.	NATHALIA VITORIA PEREIRA ARAUJO HALL	2783
HAMERSCHLAK, N.	NELSON HAMERSCHLAK	3472
HARTWEGER, H.	HARALD HARTWEGER	3413
HASSELSTROM, G.H.	GUILHERME HENRIQUE HASSELSTROM	2737

HASSELSTROM, G.H.	GUILHERME HENRIQUE HASSELSTROM	3092
HASSLOCHER-MORENO, A.M.	ALEJANDRO MARCEL HASSLOCHER-MORENO	3359
HASSLOCHER-MORENO, A.M.	ALEJANDRO MARCEL HASSLOCHER-MORENO	3404
HAUBERT, A.F.	ALISSON FELIPE HAUBERT	3117
HAUBERT, A.F.	ALISSON FELIPE HAUBERT	3254
HAUBERT, A.F.	ALISSON FELIPE HAUBERT	3368
HAYASHI, J.Y.	JACKELINNE YUKA HAYASHI	2923
HAYASHI, M.A.F.	MIRIAN A F HAYASHI	3423
HEATH, W.	WILLIAM HEATH	2989
HEINEMANN, M.B.	MARCOS BRYAN HEINEMANN	2990
HEINEMANN, M.B.	MARCOS BRYAN HEINEMANN	3010
HEINEMANN, M.B.	MARCOS BRYAN HEINEMANN	3020
HEINEMANN, M.B.	MARCOS BRYAN HEINEMANN	3281
HENNING, P.M.	PETRA MARIA HENNING	3119
HENRIQUES, M.D.G.M.D.O.	MARIA DAS GRAÇAS MULLER DE OLIVEIRA HENRIQUES	3043
HENRIQUES, P.M.	PRISCILLA MIRANDA HENRIQUES	2638
HENRIQUES, P.M.	PRISCILLA MIRANDA HENRIQUES	2715
HERINGER, L.D.S.	LUIZA DOS SANTOS HERINGER	3110
HERNANDEZ, B.	BRIANNA HERNANDEZ	3007
HERRERA, A.J.R.	ANDREA JAZEL RODRÍGUEZ HERRERA	2970
HERRERA, A.J.R.	ANDREA JAZEL RODRÍGUEZ HERRERA	3130
HERRERA, A.J.R.	ANDREA JAZEL RODRÍGUEZ HERRERA	3185
HERRERA, A.J.R.	ANDREA JAZEL RODRÍGUEZ HERRERA	3401
HERRERA, A.J.R.	ANDREA JAZEL RODRÍGUEZ HERRERA	3465
HERRMANN, F.	FÁBIO HERRMANN	2880
HESSMANN, E.	ELINE HESSMANN	3115
HIDALGO, A.R.	ADOLFO ROJAS HIDALGO	2724
HIGA, L.	LUIZA HIGA	2719
HIGA, L.	LUIZA HIGA	3150
HINGST-ZAHER, E.	ERIKA HINGST-ZAHER	2777
HIPÓLITO, C.B.G.D.A.	CAMILLY BEATRIZ GUEDES DE ANDRADE HIPÓLITO	2946
HIROSE, G.	GUILHERME HIROSE	3410
HIYANE, M.I.	MEIRE IOSHIE HIYANE	3143
HOAGLAND, B.	BRENDA HOAGLAND	3064
HOBBS, A.	ALVARO HOBBS	3178
HÖHER, J.A.	JORGE AMILTON HÖHER	3368
HONORIO, N.N.	NICOLE N.T.B.S. HONORIO	2906
HONORIO, N.N.	NICOLE N.T.B.S. HONORIO	3450
HOPPEN JUNIOR, A.	ALEXANDRE HOPPEN JUNIOR	2880
HORTA, L.	LAILA HORTA	2991
HORTA, M.D.F.M.	MARIA DE FÁTIMA MARTINS HORTA	2981
HORTA, M.D.F.M.	MARIA DE FÁTIMA MARTINS HORTA	2993
HOTTZ, E.	EUGÊNIO HOTTZ	3340
HOTTZ, E.	EUGÊNIO HOTTZ	3456
HOTTZ, E.	EUGÊNIO HOTTZ	3490
HOTTZ, E.D.	EUGENIO DAMACENO HOTTZ	3116
HOTTZ, E.D.	EUGENIO DAMACENO HOTTZ	3247
HOTTZ, E.D.	EUGENIO DAMACENO HOTTZ	3248
HOTTZ, E.D.	EUGENIO DAMACENO HOTTZ	3273
HOTTZ, E.D.	EUGENIO DAMACENO HOTTZ	3280
HOTTZ, E.D.	EUGENIO DAMACENO HOTTZ	3287
HOTTZ, E.D.	EUGENIO DAMACENO HOTTZ	3302
HOTTZ, E.D.	EUGENIO DAMACENO HOTTZ	3331
HUBNER, L.J.	LUIZA JOHANNA HUBNER	2890
HUI, E.	ENFU HUI	2804
HUMMEL, G.A.	GIULIA AMELIO HUMMEL	3126
HUMMEL, G.A.	GIULIA AMELIO HUMMEL	3388
IAMAMOTO, K.T.	KAREN TIEMI IAMAMOTO	2934
IBRAHIM, C.H.O.	CATARINA HARUMI ODA IBRAHIM	3149

IEBBA, V.	VALERIO IEBBA	3508
IGNACIO, A.	ALINE IGNACIO	2926
IGNACIO, A.	ALINE IGNACIO	3449
IKEGAMI, A.	AMANDA IKEGAMI	3093
IKENOHUCHI, Y.J.	YODA JANAINA IKENOHUCHI	2971
INOUE, M.G.S.	MATHEUS GALLARDO SOUZA INOUE	2895
INOUE, M.G.S.	MATHEUS GALLARDO SOUZA INOUE	3045
IOHAN, L.	LUKAS IOHAN	3060
IOHAN, L.	LUKAS IOHAN	3314
IOHAN, L.	LUKAS IOHAN	3364
IORIO, J.F.D.	JULIANA FORTES DI IORIO	2730
IRINEU, M.D.O.	MARA DE OLIVEIRA IRINEU	2894
ISAAC, L.	LOURDES ISAAC	2792
ISAAC, L.	LOURDES ISAAC	2858
ISAAC, L.	LOURDES ISAAC	2990
ISAAC, L.	LOURDES ISAAC	3020
ISHIMOTO, A.	ADRIENE ISHIMOTO	3465
IVALDO FILHO, .	IVALDO FILHO	3269
IZOLAN, L.B.	LUCAS BARBOSA IZOLAN	2813
IZOLAN, L.B.	LUCAS BARBOSA IZOLAN	2822
IZOLAN, L.B.	LUCAS BARBOSA IZOLAN	3116
JABOR, P.N.	PEDRO NAGIB JABOR	3483
JACAUNA, A.	ADRIANA JACAUNA	2790
JACAUNA, A.	ADRIANA JACAUNA	3028
JACOBS, T.	THOMAS JACOBS	2754
JACOBSEN, J.T.	JOHANNE T. JACOBSEN	3413
JANINI, L.M.R.	LUIZ MARIO RAMOS JANINI	2740
JANINI, L.M.R.	LUIZ MARIO RAMOS JANINI	2939
JANINI, L.M.R.	LUIZ MARIO RAMOS JANINI	3042
JANKOVIC, D.	DRAGANA JANKOVIC	2715
JANUARIO, M.E.S.	MARA ELISAMA S. JANUARIO	2802
JANUZZI, G.L.R.	GABRIELLA LOPES RICARDO JANUZZI	3248
JANUZZI, G.L.R.	GABRIELLA LOPES RICARDO JANUZZI	3331
JANUZZI, L.L.R.	LUIZA LOPES RICARDO JANUZZI	3248
JANUZZI, L.L.R.	LUIZA LOPES RICARDO JANUZZI	3287
JANUZZI, L.L.R.	LUIZA LOPES RICARDO JANUZZI	3330
JANUZZI, L.L.R.	LUIZA LOPES RICARDO JANUZZI	3331
JANUZZI, L.L.R.	LUIZA LOPES RICARDO JANUZZI	3340
JANUZZI, L.L.R.	LUIZA LOPES RICARDO JANUZZI	3342
JAQUECT, A.	ALAIN JAQUECT	3182
JEREMIAS, W.	WANDER JEREMIAS	3294
JERÔNIMO, S.M.B.	SELMA MARIA BEZERRA JERÔNIMO	3068
JORGE, R.G.A.C.	RENAN GALUZO ALVES CARVALHO JORGE	3154
JORGE, R.G.A.D.C.	RENAN GALUZO ALVES DE CARVALHO JORGE	2812
JORGE, S.A.C.	SORAIA ATTIE CALIL JORGE	3351
JUCÁ, P.M.	PALOMA MARINHO JUCÁ	3230
JULVE, J.	JOSEP JULVE	2957
JÚNIOR, A.B.D.C.	ARCHIMEDES BARBOSA DE CASTRO JÚNIOR	3085
JÚNIOR, C.M.Q.	CELSO MARTINS QUEIROZ JÚNIOR	2681
JÚNIOR, C.M.Q.	CELSO MARTINS QUEIROZ JÚNIOR	3421
JÚNIOR, C.R.D.A.	CARLOS ROBERTO DE ALMEIDA JÚNIOR	2651
JÚNIOR, H.C.D.S.	HAROLDO CID DA SILVA JÚNIOR	2778
JÚNIOR, H.C.D.S.	HAROLDO CID DA SILVA JÚNIOR	3154
JÚNIOR, J.D.	JOSÉ DONATO JÚNIOR	3076
JÚNIOR, J.O.D.A.F.	JOÃO OSCAR DE ALMEIDA FALCÃO JÚNIOR	2635
JÚNIOR, O.C.	OSWALDO CARDOSO JÚNIOR	3051
JÚNIOR, O.D.C.F.	ORLANDO DA COSTA FERREIRA JÚNIOR	2963
JÚNIOR, O.D.C.F.	ORLANDO DA COSTA FERREIRA JÚNIOR	2977
JÚNIOR, R.B.M.	RONALDO BRAGANÇA MARTINS JÚNIOR	3130

JÚNIOR, R.B.M.	RONALDO BRAGANÇA MARTINS JÚNIOR	3303
JÚNIOR, R.B.M.	RONALDO BRAGANÇA MARTINS JÚNIOR	3307
JÚNIOR, V.R.	VIRMONDES RODRIGUES JÚNIOR	2705
JÚNIOR, W.F.D.S.	WANDERSON FERREIRA DA SILVA JÚNIOR	3305
JÚNIOR, W.F.D.S.	WANDERSON FERREIRA DA SILVA JÚNIOR	3334
JUNQUEIRA, C.F.	CAROLINE FURTADO JUNQUEIRA	2632
JUNQUEIRA, C.F.	CAROLINE FURTADO JUNQUEIRA	2633
JUNQUEIRA, C.F.	CAROLINE FURTADO JUNQUEIRA	2648
JUNQUEIRA, C.F.	CAROLINE FURTADO JUNQUEIRA	3347
JUNQUEIRA, C.F.	CAROLINE FURTADO JUNQUEIRA	3509
JUSTO, G.	GRAÇA JUSTO	2891
JUSTO, G.	GRAÇA JUSTO	3005
KADAUKE, S.	STEPHAN KADAUKE	3142
KAISERLIAN, D.	DOMINQUE KAISERLIAN	2963
KALIL, A.N.	ANTÔNIO NOCCHI KALIL	2880
KALIL, J.	JORGE KALIL	2740
KALIL, J.	JORGE KALIL	3074
KALIL, J.	JORGE KALIL	3093
KALIL, J.	JORGE KALIL	3101
KALIL, J.	JORGE KALIL	3140
KALIL, J.	JORGE KALIL	3149
KALLÁS, E.G.	ESPER GEORGES KALLÁS	3362
KALLÁS, E.G.	ESPER G. KALLÁS	3476
KANASHIRO, A.	ALEXANDRE KANASHIRO	3076
KANO, F.S.	FLORA S. KANO	2666
KAPRITCHKOFF, R.T.I.	RAFAELA TAMI IKEDA KAPRITCHKOFF	2782
KARIN, M.	MICHAEL KARIN	2831
KATO, E.E.	ELLEN EMI KATO	2902
KATO, E.E.	ELLEN EMI KATO	3326
KATO, K.T.	KATHIA TERUMI KATO	3124
KATO, K.T.	KATHIA TERUMI KATO	3223
KATTAH, H.X.L.	HOXANA XAVIER LOPES KATTAH	3265
KEHDY, F.S.G.	FERNANDA S G KEHDY	2833
KELLER, A.C.	ALEXANDRE CASTRO KELLER	2765
KELLER, A.C.	ALEXANDRE CASTRO KELLER	2877
KELLER, A.C.	ALEXANDRE CASTRO KELLER	3211
KERBAUY, L.N.	LUCILA NASSIF KERBAUY	3472
KESKEY, R.	ROBERT KESKEY	2882
KESSELRING, R.	REBECCA KESSELRING	2819
KETTELHUT, I.D.C.	ISIS DO CARMO KETTELHUT	3269
KHAN, Z.	ZARA KHAN	3329
KHAWAJA, N.	NEHA KHAWAJA	2844
KHAYAT, A.S.	ANDRÉ SALIM KHAYAT	3048
KHOURI, R.	RICARDO KHOURI	2991
KHOURI, R.	RICARDO KHOURI	3125
KHOURI, R.	RICARDO KHOURI	3318
KHOURI, R.	RICARDO KHOURI	3508
KIMMEMGS, T.N.	THAIS NASCIMENTO KIMMEMGS	3126
KIMMEMGS, T.N.	THAIS NASCIMENTO KIMMEMGS	3388
KINKER, G.S.	GABRIELA SARTI KINKER	2827
KINKER, G.S.	GABRIELA SARTI KINKER	2841
KINKER, G.S.	GABRIELA SARTI KINKER	3029
KINKER, G.S.	GABRIELA SARTI KINKER	3039
KINKER, G.S.	GABRIELA SARTI KINKER	3094
KINKER, G.S.	GABRIELA SARTI KINKER	3171
KINKER, G.S.	GABRIELA SARTI KINKER	3309
KINKER, G.S.	GABRIELA SARTI KINKER	3392
KITOKO, J.Z.	JAMIL ZOLA KITOKO	3086
KOH, C.C.	CAROLINA CATTONI KOH	2972

KOH, C.C.	CAROLINA CATTONI KOH	3028
KOH, C.C.	CAROLINA CATTONI KOH	3234
KOHLER, H.F.	HUGO FONTAN KOHLER	3317
KOIKE, G.	GABRIELA KOIKE	3149
KOLLIGUNDLA, L.P.	LAKSHMI P KOLLIGUNDLA	3506
KONT, K.	KEN KONT	2853
KONZEN, L.S.R.	LAURA SOLANO REINEHR KONZEN	3092
KOSTERS, A.	ASTRID KOSTERS	2789
KOVTONYUK, L.	LARISA KOVTONYUK	2926
KOWALSKI, L.P.	LUIZ PAULO KOWALSKI	3317
KRETZER, I.F.	IARA FABRICIA KRETZER	3300
KUBES, P.	PAUL KUBES	2853
KUCYKOWICZ, S.	STEPHANIE KUCYKOWICZ	2844
KUIL, L.D.M.	LARISSA DE MELO KUIL	2842
KUPEK, E.	EMIL KUPEK	3449
KURTENBACH, E.	ELEONORA KURTENBACH	3393
LABATE, C.A.	CARLOS ALBERTO LABATE	2946
LABATE, J.D.L.	JULIANA DA LUZ LABATE	2765
LABATE, J.D.L.	JULIANA DA LUZ LABATE	2877
LABEAUD, A.D.	ANGELLE DESIREE LABEAUD	2644
LABEAUD, A.D.	ANGELLE DESIREE LABEAUD	2652
LABOURBE, E.	EMMA LABOURBE	2960
LACERDA, G.D.S.	GILMAR DE SOUZA LACERDA	3006
LACERDA, M.	MARCUS LACERDA	2991
LACO, A.C.S.	ANA CAROLINA SHUAN LACO	3272
LAGE, A.C.P.	ANNA CAROLINA PINHEIRO LAGE	2653
LAGE, A.C.P.	ANNA CAROLINA PINHEIRO LAGE	2654
LAGO, A.	ALEXSANDRO LAGO	3125
LAGO, F.G.	FERNANDA GOMES LAGO	3001
LALLO, M.A.	MARIA ANETE LALLO	2879
LAMBRIS, J.D.	JOHN D. LAMBRIS	3319
LAMOGLIA, A.	ANDREIA LAMOGLIA	3404
LAMOGLIA, A.	ANDREIA LAMOGLIA	3505
LAMOGLIA, A.S.A.	ANTONIETA SANTOS ANDRADE LAMOGLIA	3454
LAMOGLIA, A.S.A.	ANTONIETA SANTOS ANDRADE LAMOGLIA	3486
LAMOGLIA, A.S.A.	ANTONIETA SANTOS ANDRADE LAMOGLIA	3493
LAMOGLIA, A.S.A.	ANTONIETA SANTOS ANDRADE LAMOGLIA	3495
LAMOGLIA, A.S.A.	ANTONIETA SANTOS ANDRADE LAMOGLIA	3497
LAMOGLIA, A.S.A.	ANTONIETA SANTOS ANDRADE LAMOGLIA	3516
LANCASTER, J.N.	JESSICA N LANCASTER	2935
LANDAY, A.	ALAN LANDAY	3362
LANGER, R.S.	RAFAEL SAAVEDRA LANGER	2676
LANNES-VIEIRA, J.	JOSELI LANNES-VIEIRA	3359
LANNES-VIEIRA, J.	JOSELI LANNES-VIEIRA	3404
LARA, F.A.	FLÁVIO ALVES LARA	2986
LISSO, J.A.L.	JORDY ALEXANDER LARCO LISSO	2895
LISSO, J.A.L.	JORDY ALEXANDER LARCO LISSO	3045
LISSOUNSKAIA, E.	ELENA LISSOUNSKAIA	2986
LAWRES, L.	LAUREN LAWRES	2719
LAYDEN, B.T.	BRIAN T. LAYDEN	2843
LAZETIC, V.	VLADIMIR LAZETIC	3066
LEAL, A.M.L.D.C.	AMANDA MARIA LAGO DE CASTRO LEAL	2775
LEAL, A.M.L.D.C.	AMANDA MARIA LAGO DE CASTRO LEAL	3250
LEAL, A.M.L.D.C.	AMANDA MARIA LAGO DE CASTRO LEAL	3298
LEAL, A.M.L.D.C.	AMANDA MARIA LAGO DE CASTRO LEAL	3464
LEAL, M.	MANUELA LEAL	3095
LEAL, R.F.	RAQUEL FRANCO LEAL	3067
LEAL, V.N.C.	VINÍCIUS NUNES CORDEIRO LEAL	2767
LEANDRO, M.D.O.	MAÍSA DE OLIVEIRA LEANDRO	2755

LEANDRO, M.D.O.	MAÍSA DE OLIVEIRA LEANDRO	3088
LEANDRO, M.D.O.	MAÍSA DE OLIVEIRA LEANDRO	3097
LEANDRO, M.D.O.	MAÍSA DE OLIVEIRA LEANDRO	3170
LEANDRO, M.D.O.	MAÍSA DE OLIVEIRA LEANDRO	3384
LEANDRO, M.D.O.	MAÍSA DE OLIVEIRA LEANDRO	3408
LEÃO, E.R.	ELISETH RIBEIRO LEÃO	2777
LEBANI, P.L.	PIETRO LUIGI LEBANI	2975
LEIRIA, L.O.S.	LUIZ OSÓRIO SILVEIRA LEIRIA	3111
LEIRIA, L.O.S.	LUIZ OSÓRIO SILVEIRA LEIRIA	3269
LEIRIA, L.O.S.	LUIZ OSÓRIO SILVEIRA LEIRIA	3270
LEIRIA, L.O.S.	LUIZ OSÓRIO SILVEIRA LEIRIA	3320
LEITE, A.C.C.B.	ANA CLAUDIA CELESTINO BEZERRA LEITE	2814
LEITE, A.C.C.B.	ANA CLAUDIA CELESTINO BEZERRA LEITE	3145
LEITE, A.C.C.B.	ANA CLAUDIA CELESTINO BEZERRA LEITE	3425
LEITE, G.G.F.	GIUSEPPE GIANINI FIGUEIREDO LEITE	2899
LEITE, G.G.F.	GIUSEPPE GIANINI FIGUEIREDO LEITE	2923
LEITE, H.M.	HELOINE M LEITE	3219
LEITE, J.A.	JEFFERSON A. LEITE	3130
LEITE, J.A.	JEFFERSON A. LEITE	3132
LEITE, J.A.	JEFFERSON ANTÔNIO LEITE	3143
LEITE, L.C.D.C.	LUCIANA CEZAR DE CERQUEIRA LEITE	2761
LEITE, L.C.D.C.	LUCIANA CEZAR DE CERQUEIRA LEITE	2771
LEITE, L.C.D.C.	LUCIANA CEZAR DE CERQUEIRA LEITE	2791
LEITE, L.C.D.C.	LUCIANA CEZAR DE CERQUEIRA LEITE	2793
LEITE, L.D.C.T.	LAYSLA DE CAMPOS TOLEDO LEITE	2875
LEITE, N.P.D.S.	NATHÁLIA PEREIRA DA SILVA LEITE	3513
LEME, A.F.P.	ADRIANA FRANCO PAES LEME	2733
LEME, V.D.C.	VICTÓRIA DE CASTRO LEME	3468
LEMEIRE, K.	KELLY LEMEIRE	3115
LEMONS, A.P.D.A.	ANA PATRICIA DE ALMEIDA LEMOS	2810
LEMONS, A.P.D.A.	ANA PATRICIA DE ALMEIDA LEMOS	2995
LEMONS, A.P.D.A.	ANA PATRICIA DE ALMEIDA LEMOS	3006
LEMONS, A.P.D.A.	ANA PATRICIA DE ALMEIDA LEMOS	3136
LEMONS, J.A.D.S.	JULIANA ALINE DE SOUZA LEMOS	3050
LEMONS, M.M.M.	MARTA MARIA MOREIRA LEMOS	3029
LEMONS, M.M.M.	MARTA MARIA MOREIRA LEMOS	3171
LEMONS, P.B.D.M.	PAMELA BEATRIZ DE MENEZES LEMOS	2649
LEOCATA, B.	BEATRIZ LEOCATA	3126
LEONEL, G.M.	GISELE MANFFRENATTI LEONEL	3399
LEONEL, T.B.	THYAGO BISPO LEONEL	3319
LEPIQUE, A.P.	ANA PAULA LEPIQUE	2752
LEPIQUE, A.P.	ANA PAULA LEPIQUE	2774
LEPIQUE, A.P.	ANA PAULA LEPIQUE	2889
LEPIQUE, A.P.	ANA PAULA LEPIQUE	2895
LEPIQUE, A.P.	ANA PAULA LEPIQUE	2945
LEPIQUE, A.P.	ANA PAULA LEPIQUE	2961
LEPIQUE, A.P.	ANA PAULA LEPIQUE	2966
LEPIQUE, A.P.	ANA PAULA LEPIQUE	3045
LEPIQUE, A.P.	ANA PAULA LEPIQUE	3292
LERNER, U.H.	ULF HOLGER LERNER	3119
LESSA, A.C.M.	ALANA CONCEIÇÃO MAIA LESSA	2871
LESSA, A.C.M.	ALANA CONCEIÇÃO MAIA LESSA	2887
LESSA, A.C.M.	ALANA CONCEIÇÃO MAIA LESSA	2910
LESSA, A.C.M.	ALANA CONCEIÇÃO MAIA LESSA	3025
LESSA, A.C.M.	ALANA CONCEIÇÃO MAIA LESSA	3520
LEUNG, C.	CAROL LEUNG	2929
LEVINSHON, J.	JONATHAN LEVINSHON	3506
LI, S.	SAMUEL LI	3066
LIANG, Y.	YISHA LIANG	3027

LIANI, S.A.C.	SOFIA AQUINO CARDOSO LIANI	3232
LIANI, S.A.C.	SOFIA AQUINO CARDOSO LIANI	3418
LIANI, S.A.C.	SOFIA AQUINO CARDOSO LIANI	3484
LIBERA, A.M.M.P.D.	ALICE MARIA MELVILLE PAIVA DELLA LIBERA	3010
LIBERA, A.M.M.P.D.	ALICE MARIA MELVILLE PAIVA DELLA LIBERA	3281
LÍBERO, L.O.	LAURA ORDONHO LÍBERO	3255
LIDIO, A.V.	ADRIELLY VARGAS LIDIO	3118
LIECHTI, T.	THOMAS LIECHTI	2715
LIMA JUNIOR, J.D.C.	JOSUE DA COSTA LIMA JUNIOR	2743
LIMA JUNIOR, J.D.C.	JOSUE DA COSTA LIMA JUNIOR	2893
LIMA JUNIOR, J.D.C.	JOSUE DA COSTA LIMA JUNIOR	2940
LIMA JUNIOR, J.D.C.	JOSUE DA COSTA LIMA JUNIOR	3050
LIMA, A.A.L.	ANA ALICE LEMOS LIMA	3087
LIMA, A.C.	ANA CAROLINA LIMA	2679
LIMA, A.C.P.	ALANA CONCEIÇÃO PEREIRA LIMA	3373
LIMA, A.C.P.	ALANA CONCEIÇÃO PEREIRA LIMA	3406
LIMA, A.C.P.	ALANA CONCEIÇÃO PEREIRA LIMA	3441
LIMA, A.C.P.	ALANA CONCEIÇÃO PEREIRA LIMA	3504
LIMA, A.E.D.S.	ANA ESTHER DE SOUZA LIMA	2756
LIMA, A.E.D.S.	ANA ESTHER DE SOUZA LIMA	3267
LIMA, B.G.C.	BRUNA GENISA COSTA LIMA	3315
LIMA, C.N.C.	CARLOS NEANDRO CORDEIRO LIMA	3124
LIMA, C.N.C.	CARLOS NEANDRO CORDEIRO LIMA	3139
LIMA, C.N.C.	CARLOS NEANDRO CORDEIRO LIMA	3223
LIMA, E.	EDUARDA LIMA	3183
LIMA, G.D.M.	GEOVANNI DE MORAIS LIMA	2734
LIMA, G.D.M.	GEOVANNI DE MORAIS LIMA	2944
LIMA, G.D.M.	GEOVANNI DE MORAIS LIMA	3067
LIMA, G.T.D.C.F.	GUSTAVO TAVARES DA COSTA FIUZA LIMA	3426
LIMA, H.F.	HIGOR FRANÇA LIMA	3006
LIMA, I.D.	ISRAEL DINIZ LIMA	2738
LIMA, I.D.	ISRAEL DINIZ LIMA	3418
LIMA, I.V.D.A.	ISABEL VIEIRA DE ASSIS LIMA	2847
LIMA, J.E.N.	JULIANA ECHEVARRIA N. LIMA	2795
LIMA, J.E.N.	JULIANA ECHEVARRIA N. LIMA	2796
LIMA, J.E.N.	JULIANA ECHEVARRIA N. LIMA	2814
LIMA, J.E.N.	JULIANA ECHEVARRIA N. LIMA	2818
LIMA, J.E.N.	JULIANA ECHEVARRIA N. LIMA	3150
LIMA, J.E.N.	JULIANA ECHEVARRIA N. LIMA	3471
LIMA, K.	KARINA LIMA	2804
LIMA, K.C.N.	KELY CAMPOS NAVEGANTES LIMA	3522
LIMA, L.	LUCIANO LIMA	2777
LIMA, L.K.S.C.	LÍVIA KAORI SEINO CORRÊA LIMA	3459
LIMA, L.M.	LÍDIA MOREIRA LIMA	3246
LIMA, L.R.	LUAN ROCHA LIMA	3494
LIMA, M.A.	MARCO ANTONIO LIMA	2814
LIMA, M.A.	MARCO ANTONIO LIMA	3145
LIMA, M.A.	MARCO ANTONIO LIMA	3425
LIMA, M.J.T.	MARIA JÚLIA TÔRRES LIMA	3248
LIMA, M.J.T.	MARIA JÚLIA TÔRRES LIMA	3287
LIMA, M.J.T.	MARIA JÚLIA TÔRRES LIMA	3330
LIMA, M.J.T.	MARIA JÚLIA TÔRRES LIMA	3340
LIMA, M.J.T.	MARIA JÚLIA TÔRRES LIMA	3458
LIMA, M.P.O.	MARIA PAULA OLIVEIRA LIMA	2855
LIMA, M.P.O.	MARIA PAULA OLIVEIRA LIMA	3226
LIMA, M.P.O.	MARIA PAULA OLIVEIRA LIMA	3236
LIMA, M.R.	MARLUCY RODRIGUES LIMA	3219
LIMA, M.R.D.	MARIA REGINA D'IMPÉRIO LIMA	2935
LIMA, M.R.D.	MARIA REGINA D'IMPÉRIO LIMA	2989

LIMA, M.R.D.	MARIA REGINA D'IMPÉRIO LIMA	3008
LIMA, M.R.D.	MARIA REGINA D'IMPÉRIO LIMA	3230
LIMA, M.R.D.	MARIA REGINA D'IMPÉRIO LIMA	3325
LIMA, M.V.D.S.	MARCELLY VITÓRIA DA SILVA LIMA	2874
LIMA, M.V.D.S.	MARCELLY VITÓRIA DA SILVA LIMA	2911
LIMA, V.D.S.	VALFRAN DA SILVA LIMA	2737
LIMA, V.D.S.	VALFRAN DA SILVA LIMA	3092
LIMA, V.D.S.	VALFRAN DA SILVA LIMA	3322
LIMA-GOMES, P.D.S.	PHILLIPE DE SOUZA LIMA-GOMES	2898
LIMA-JÚNIOR, D.D.S.	DJALMA DE SOUZA LIMA-JÚNIOR	3158
LIMA-JÚNIOR, D.D.S.	DJALMA DE SOUZA LIMA-JÚNIOR	3452
LIMA-JÚNIOR, D.D.S.	DJALMA DE SOUZA LIMA-JÚNIOR	3511
LIMA-JUNIOR, J.D.C.	JOSUÉ DA COSTA LIMA-JUNIOR	3355
LIMA-JUNIOR, J.D.C.	JOSUÉ DA COSTA LIMA-JUNIOR	3356
LINO, K.	KATIA LINO	3006
LINS, M.	MARVIN LINS	3254
LINS, M.P.	MARVIN PAULO LINS	3353
LIONE, V.D.O.F.	VIVIANE DE OLIVEIRA FREITAS LIONE	3523
LIRA, F.S.	FÁBIO S. LIRA	2826
LIRA, F.S.	FÁBIO S. LIRA	2866
LISBOA, L.F.T.	LARA FABIAN TEIXEIRA LISBOA	3522
LISBOA, S.F.	SABRINA F. LISBOA	3188
LISBOA, S.F.	SABRINA F. LISBOA	3307
LITTMAN, D.	DAN LITTMAN	3086
LIU, A.	AIZHU LIU	3161
LIU, T.	TONG LIU	3329
LOIOLA, L.A.	LORENA ALCANTARA LOIOLA	3057
LOIOLA, L.H.M.	LUIS HENRIQUE MAIA LOIOLA	2914
LOIOLA, L.H.M.	LUIS HENRIQUE MAIA LOIOLA	2997
LOMBA, G.S.B.	GUILHERME SCHITTINE BEZERRA LOMBA	2998
LOPES, A.L.R.B.	ANTÔNIO LUIZ RIBEIRO BOACHAT LOPES	3383
LOPES, C.D.A.	CAMILA DE ALMEIDA LOPES	3452
LOPES, C.D.A.	CAMILA DE ALMEIDA LOPES	3511
LOPES, F.	FELIPE LOPES	3130
LOPES, F.T.	FELIPE TEIXEIRA LOPES	3185
LOPES, G.D.S.	GISELE DA SILVA LOPES	3058
LOPES, G.L.D.M.M.	GIOVANNA LEIROZ DE MELLO MUSSE LOPES	3306
LOPES, L.B.	LUÍSA BELMONTE LOPES	3186
LOPES, L.M.S.	LUCIANA MARIA SILVA LOPES	2716
LOPES, L.M.S.	LUCIANA MARIA SILVA LOPES	2717
LOPES, M.	MILENA LOPES	3187
LOPES, M.	MILENA LOPES	3411
LOPES, M.D.F.	MARCELA DE FREITAS LOPES	2892
LOPES, M.F.S.	MARYA FERNANDA SANTOS LOPES	2707
LOPES, M.R.G.	MILENA REGINA GOMES LOPES	3174
LOPES, M.R.G.	MILENA REGINA GOMES LOPES	3184
LOPES, M.R.G.	MILENA REGINA GOMES LOPES	3249
LOPES, P.D.O.	PAOLA DE OLIVEIRA LOPES	2862
LOPES, P.D.O.	PAOLA DE OLIVEIRA LOPES	2868
LOPES, P.O.	PAOLA OLIVEIRA LOPES	2898
LOPES, P.O.	PAOLA OLIVEIRA LOPES	3059
LOPES, P.O.	PAOLA OLIVEIRA LOPES	3403
LOPES, P.O.	PAOLA OLIVEIRA LOPES	3489
LOPES, T.B.	THICIANY BLENER LOPES	2663
LOPES, V.D.S.	VICTOR DOS SANTOS LOPES	2994
LOPES, V.D.S.	VICTOR DOS SANTOS LOPES	2996
LOPES, V.D.S.	VICTOR DOS SANTOS LOPES	3021
LOPES, V.D.S.	VICTOR DOS SANTOS LOPES	3035
LOPEZ-CAMACHO, C.	CESAR LOPEZ-CAMACHO	2743

LÓPEZ-GONZÁLEZ, M.Á.	MARÍA ÁNGELES LÓPEZ-GONZÁLEZ	3139
LOREDO, I.C.	ISADORA CUNHA LOREDO	3109
LORGA, A.C.M.	ANA CAROLINA MIKEJEVS LORGA	3020
LOURENÇO, A.A.	ALICE APARECIDA LOURENÇO	2692
LOURENÇO, A.J.	ADELINA JUNIA LOURENÇO	2756
LOURENÇO, A.J.	ADELINA JUNIA LOURENÇO	3267
LOURENÇO, A.J.	ADELINA JUNIA LOURENÇO	3294
LOURENZONI, M.R.	MARCOS ROBERTO LOURENZONI	3135
LOURES, F.V.	FLÁVIO VIEIRA LOURES	3515
LUBIANA, T.	TIAGO LUBIANA	2935
LUCAS, C.	CAROLINA LUCAS	2719
LUCAS, J.M.	JACKELINE MARINO LUCAS	3303
LUCAS, J.M.	JACKELINE MARINO LUCAS	3307
LUCAS, J.M.	JACKELINE MARINO LUCAS	3381
LUCAS, L.M.B.	LARISSA MENDES BARBOSA LUCAS	2657
LUCAS, L.M.B.	LARISSA MENDES BARBOSA LUCAS	2675
LUCAS, L.M.B.	LARISSA MENDES BARBOSA LUCAS	3434
LUCAS, L.M.B.	LARISSA MENDES BARBOSA LUCAS	3451
LUCINDA, P.P.D.	PEDRO PAULO DINIZ LUCINDA	2972
LUCINDA, P.P.D.	PEDRO PAULO DINIZ LUCINDA	2976
LUCINDA, P.P.D.	PEDRO PAULO DINIZ LUCINDA	3026
LUCINDA, P.P.D.	PEDRO PAULO DINIZ LUCINDA	3234
LUDWIG, R.G.	RAISSA GUIMARÃES LUDWIG	3067
LUDWIG, R.G.	RAISSA GUIMARÃES LUDWIG	3076
LUGON, J.R.	JOCEMIR RONALDO LUGON	2816
LUGON, J.R.	JOCEMIR RONALDO LUGON	2998
LULA, I.S.	IVANA SILVA LULA	2686
LUNA, N.	NATALIA LUNA	2725
LUZ, E.	ESTELA LUZ	3362
LUZ, Y.D.S.	YASMIN DA SILVA LUZ	3156
LYRA, R.	RICARDO LYRA	2833
M. FILHO, O.A.	OLINDO ASSIS M. FILHO	2693
MACCIONI, M.	MARIANA MACCIONI	2957
MACÊDO, B.D.G.	BRUNA DE GOIS MACÊDO	2935
MACEDO, G.C.	GILSON COSTA MACEDO	3081
MACEDO, G.C.	GILSON COSTA MACEDO	3082
MACEDO, G.C.	GILSON COSTA MACEDO	3102
MACEDO, G.C.	GILSON COSTA MACEDO	3116
MACEDO, G.C.	GILSON COSTA MACEDO	3147
MACEDO, G.C.	GILSON COSTA MACEDO	3162
MACEDO, G.C.	GILSON COSTA MACEDO	3203
MACEDO, G.C.	GILSON COSTA MACEDO	3213
MACÊDO, P.E.F.	PAULO ESTEVÃO FERREIRA MACÊDO	2882
MACÊDO, P.E.F.	PAULO ESTEVÃO FERREIRA MACÊDO	2920
MACÊDO, P.E.F.	PAULO ESTEVÃO FERREIRA MACÊDO	3004
MACÊDO, P.E.F.	PAULO ESTEVÃO FERREIRA MACÊDO	3252
MACÊDO, P.E.F.	PAULO ESTEVÃO FERREIRA MACÊDO	3487
MACHADO, A.A.D.S.	AMANDA APARECIDA DA SILVA MACHADO	3521
MACHADO, A.B.F.	ALESSANDRA BARBOSA FERREIRA MACHADO	3147
MACHADO, C.B.	CAIO BEZERRA MACHADO	2861
MACHADO, C.B.	CAIO BEZERRA MACHADO	2959
MACHADO, C.B.	CAIO BEZERRA MACHADO	2969
MACHADO, C.B.	CAIO BEZERRA MACHADO	3016
MACHADO, C.E.C.	CARLA ELIZABETH CUNHA MACHADO	2641
MACHADO, C.E.C.	CARLA ELIZABETH CUNHA MACHADO	2658
MACHADO, C.M.M.	CLENI MARA MARZOCCHI MACHADO	2737
MACHADO, C.M.M.	CLENI MARA MARZOCCHI MACHADO	3092
MACHADO, C.M.M.	CLENI MARA MARZOCCHI MACHADO	3357
MACHADO, C.M.M.	CLENI MARA MARZOCCHI MACHADO	3412

MACHADO, F.S.	FABIANA SIMÃO MACHADO	2651
MACHADO, F.S.	FABIANA SIMÃO MACHADO	2676
MACHADO, F.S.	FABIANA SIMÃO MACHADO	2698
MACHADO, F.S.	FABIANA SIMÃO MACHADO	2699
MACHADO, F.S.	FABIANA SIMÃO MACHADO	2701
MACHADO, F.S.	FABIANA SIMÃO MACHADO	2702
MACHADO, F.S.	FABIANA SIMÃO MACHADO	3470
MACHADO, J.C.	JOÃO CARLOS MACHADO	3109
MACHADO, L.	LUCAS MACHADO	3243
MACHADO, L.F.	LETICIA FERREIRA MACHADO	2834
MACHADO, L.F.	LETICIA FERREIRA MACHADO	2967
MACHADO, L.F.	LETICIA FERREIRA MACHADO	3081
MACHADO, L.F.	LETICIA FERREIRA MACHADO	3116
MACHADO, M.A.	MARCO ANTÔNIO MACHADO	2906
MACHADO, M.A.	MARCO ANTÔNIO MACHADO	3100
MACHADO, M.A.	MARCO ANTÔNIO MACHADO	3450
MACHADO, M.P.	MICHELE P. MACHADO	3202
MACHADO, M.S.G.	MELISSA SANTANA GONSALEZ MACHADO	2766
MACHADO, P.R.	PAULO ROBERTO MACHADO	3370
MACHADO, P.R.	PAULO ROBERTO MACHADO	3432
MACHADO, R.L.D.	RICARDO LUIZ DANTAS MACHADO	3355
MACHADO, S.A.	SABRINA AZEVEDO MACHADO	3108
MACHADO, S.A.	SABRINA AZEVEDO MACHADO	3313
MACHADO, T.L.	THIAGO LAZARI MACHADO	2812
MACHADO, T.L.	THIAGO LAZARI MACHADO	3154
MACIEL, E.M.A.	ESTHER MARQUES ALVES MACIEL	3474
MACIEL, L.D.O.	LETICIA DE OLIVEIRA MACIEL	3357
MACIEL, L.D.O.	LETICIA DE OLIVEIRA MACIEL	3412
MADEIRA, J.C.C.	JULIANA CRISTINA COSTA MADEIRA	3157
MADEIRA, J.C.C.	JULIANA CRISTINA COSTA MADEIRA	3465
MADEIRA, M.F.M.	MILA FERNANDES MOREIRA MADEIRA	2657
MADEIRA, R.P.	RAFAEL PEDRO MADEIRA	3074
MADI, K.	KALIL MADI	3109
MADRUGA, J.V.R.	JOSÉ VALDEZ RAMALHO MADRUGA	3362
MAELFAIT, J.	JONATHAN MAELFAIT	3115
MAGALDI, K.M.L.	KIMBERLYN MARCELY LOPES MAGALDI	3501
MAGALHÃES, A.S.	ANDRÉA SANTOS MAGALHÃES	3192
MAGALHÃES, G.S.	GERALDO SANTANA MAGALHÃES	3218
MAGALHÃES, G.S.	GERALDO SANTANA MAGALHÃES	3277
MAGALHÃES, H.I.F.	HEMERSON IURY FERREIRA MAGALHÃES	3341
MAGALHÃES, K.G.	KELLY GRACE MAGALHÃES	3001
MAGALHÃES, K.G.	KELLY GRACE MAGALHÃES	3044
MAGALHÃES, K.G.	KELLY GRACE MAGALHÃES	3069
MAGALHÃES, K.G.	KELLY GRACE MAGALHÃES	3313
MAGALHÃES, M.S.	MYLENA SILVA MAGALHÃES	3307
MAGALHÃES, V.D.S.	VITÓRIA DE SENA MAGALHÃES	3522
MAGALHÃES, V.R.M.	VIRGÍNIA REZENDE MAYRINK MAGALHÃES	2756
MAGALHÃES, V.R.M.	VIRGÍNIA REZENDE MAYRINK MAGALHÃES	3267
MAGALHÃES-GAMA, F.	FÁBIO MAGALHÃES-GAMA	3395
MAGALLANES, S.L.	SOFÍA LAGOS MAGALLANES	2957
MAGUETA, E.	ERICA MAGUETA	3219
MAIA, B.F.F.	BRENO FRANCISCO FIGUEIREDO MAIA	3032
MAIA, I.	ISRAEL MAIA	3449
MAIA, L.C.R.	LUCIANA CONDE RODRIGUES MAIA	2719
MAIA, L.C.R.	LUCIANA CONDE RODRIGUES MAIA	2724
MAIA, L.C.R.	LUCIANA CONDE RODRIGUES MAIA	2941
MAIA, L.C.R.	LUCIANA CONDE RODRIGUES MAIA	2977
MAIA, L.C.R.	LUCIANA CONDE RODRIGUES MAIA	3107
MAIA, L.C.R.	LUCIANA CONDE RODRIGUES MAIA	3178

MAIA, L.C.R.	LUCIANA CONDE RODRIGUES MAIA	3502
MAIA, M.D.L.D.S.	MARIA DE LOURDES DE SOUZA MAIA	2649
MAIA, M.D.L.D.S.	MARIA DE LOURDES DE SOUZA MAIA	3184
MAIA, M.D.L.D.S.	MARIA DE LOURDES DE SOUZA MAIA	3243
MAIA, M.D.L.D.S.	MARIA DE LOURDES DE SOUZA MAIA	3249
MAIA, N.S.R.	NÍCOLAS SÁ RODRIGUES MAIA	3044
MAIA, R.M.	RENATA MALACHINI MAIA	3404
MAIELLO, M.S.	MANUELA SAIRRE MAIELLO	3307
MAILHO-FONTANA, P.L.	PEDRO LUIZ MAILHO-FONTANA	3225
MAINI, M.	MALA MAINI	2844
MAIO, K.T.	KARINA TOZATO MAIO	3410
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2643
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2645
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2647
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2664
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2673
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2676
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2677
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2678
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2679
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2683
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2684
MAIOLI, T.U.	TATIANI UCELI MAIOLI	2766
MAIOLI, T.U.	TATIANI UCELI MAIOLI	3250
MALAK, S.A.	SARA ABDEL MALAK	2929
MALHEIRO, A.	ADRIANA MALHEIRO	3395
MALTEZ, F.	FERNANDO MALTEZ	2719
MAMIGONIAN, O.	OHANEZ MAMIGONIAN	3288
MANCHINE, J.P.	JULIA PERIN MANCHINE	3313
MANCINI, M.C.B.	MARIA CLARA BERTORELLI MANCINI	3280
MANCINI, M.C.B.	MARIA CLARA BERTORELLI MANCINI	3302
MANFRO, W.F.P.	WÂNIA FERRAZ PEREIRA MANFRO	2822
MANHÃES, L.D.S.B.	LUCIANA DOS SANTOS BARROS MANHÃES	2989
MANHÃES, L.D.S.B.	LUCIANA DOS SANTOS BARROS MANHÃES	3008
MANHÃES, L.D.S.B.	LUCIANA DOS SANTOS BARROS MANHÃES	3230
MANHÃES, L.D.S.B.	LUCIANA DOS SANTOS BARROS MANHÃES	3325
MANHÃES, N.C.D.S.	NAIARA CARLA DOS SANTOS MANHÃES	3346
MANSUR, D.S.	DANIEL SANTOS MANSUR	3288
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	2949
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3053
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3103
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3127
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3135
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3144
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3152
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3190
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3242
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3389
MARANHÃO, A.Q.	ANDRÉA QUEIROZ MARANHÃO	3424
MARANINCHI, R.S.	ROBERTA SILVEIRA MARANINCHI	3016
MARCELINO, T.F.	THAIS FLORIANO MARCELINO	3119
MARCHIORO, S.B.	SILVANA BEUTINGER MARCHIORO	3282
MARCON, B.H.	BRUNA HILZENDEGER MARCON	2860
MARCONDES, Y.L.	YASMIM LEMLE MARCONDES	2888
MARGINEAN, D.	DENISSE MARGINEAN	2932
MARIA, D.A.	DURVANEI AUGUSTO MARIA	3351
MARIANO, R.M.D.S.	REYSLA MARIA DA SILVEIRA MARIANO	3293
MARIANO, R.M.D.S.	REYSLA MARIA DA SILVEIRA MARIANO	3314
MARIANO, R.M.D.S.	REYSLA MARIA DA SILVEIRA MARIANO	3364

MARICATO, J.T.	JULIANA TERZI MARICATO	2740
MARINA, C.L.F.	CLARA LUNA FREITAS MARINA	3079
MARINHO, A.I.	ANANDA ISIS MARINHO	2871
MARINHO, A.I.	ANANDA ISIS MARINHO	2887
MARINHO, A.I.	ANANDA ISIS MARINHO	2910
MARINHO, A.I.	ANANDA ISIS MARINHO	3025
MARINHO, A.I.	ANANDA ISIS MARINHO	3075
MARINHO, A.I.	ANANDA ISIS MARINHO	3156
MARINHO, A.I.	ANANDA ISIS MARINHO	3318
MARINHO, A.I.	ANANDA ISIS MARINHO	3520
MARINHO, A.K.B.B.	ANA KAROLINA BARRETO BERSELLI MARINHO	3371
MARINHO, C.R.F.	CLÁUDIO ROMERO FARIAS MARINHO	3008
MARINHO, F.A.V.	FABIO ANTONIO VITARELLI MARINHO	2828
MARINHO, F.V.	FABIO VITARELLI MARINHO	2781
MARINO, A.P.	ANA PAULA MARINO	2715
MARINS, J.D.G.	JUAN DA GAMA MARINS	2897
MARINS, J.D.G.	JUAN DA GAMA MARINS	3099
MARINS, J.D.G.	JUAN DA GAMA MARINS	3132
MARINS, J.D.G.	JUAN DA GAMA MARINS	3138
MARINS, J.D.G.	JUAN DA GAMA MARINS	3195
MARINS, J.D.G.	JUAN DA GAMA MARINS	3238
MARINS, J.D.G.	JUAN DA GAMA MARINS	3241
MARINS, J.D.G.	JUAN DA GAMA MARINS	3256
MARMELLO, B.O.	BÁRBARA OLIVEIRA MARMELLO	2933
MARON-GUTIERREZ, T.	TATIANA MARON-GUTIERREZ	3036
MARQUES, C.B.	CAROLINA BRANDI MARQUES	2834
MARQUES, C.G.D.S.	CAIO GABRIEL DA SILVA MARQUES	3289
MARQUES, C.G.D.S.	CAIO GABRIEL DA SILVA MARQUES	3353
MARQUES, C.G.D.S.	CAIO GABRIEL DA SILVA MARQUES	3488
MARQUES, D.P.D.A.	DAISYMARA PRISCILA DE ALMEIDA MARQUES	2693
MARQUES, J.D.D.P.C.	JOÃO DUARTE DE PAULA COUTINHO MARQUES	3135
MARQUES, J.D.D.P.C.	JOÃO DUARTE DE PAULA COUTINHO MARQUES	3424
MARQUES, L.F.	LARISSA FONSECA MARQUES	2752
MARQUES, M.F.S.	MICHELE FLÁVIA SOUSA MARQUES	3010
MARQUES, M.J.	MARCOS JOSÉ MARQUES	3246
MARQUES, R.F.	RODOLFO FERREIRA MARQUES	2740
MARQUES, R.F.	RODOLFO FERREIRA MARQUES	3149
MARQUES, R.M.	RENATO MORETTI MARQUES	3314
MARQUES, R.M.	RENATO MORETTI MARQUES	3364
MARQUES, T.M.	TIAGO MARTINS MARQUES	2653
MARQUES, T.M.	TIAGO MARTINS MARQUES	2654
MARTIM, K.L.G.	KAUÃ LEONARDO GALVÃO MARTIM	3492
MARTINELLI, M.	MATHEUS MARTINELLI	3060
MARTINELLI, M.	MATHEUS MARTINELLI	3314
MARTINELLI, M.	MATHEUS MARTINELLI	3364
MARTINEZ, G.A.	GRACIA APARECIDA MARTINEZ	2854
MARTÍNEZ-CONDE, C.I.Á.	CLAUDIA ISABEL ÁLVAREZ MARTÍNEZ-CONDE	3074
MARTINS, A.D.S.	ARIELE DA SILVA MARTINS	3278
MARTINS, A.J.E.	ANA JULIA ESTUMANO MARTINS	2772
MARTINS, A.J.E.	ANA JULIA ESTUMANO MARTINS	2775
MARTINS, A.J.E.	ANA JULIA ESTUMANO MARTINS	2809
MARTINS, A.J.E.	ANA JULIA ESTUMANO MARTINS	2829
MARTINS, A.J.E.	ANA JULIA ESTUMANO MARTINS	2832
MARTINS, A.J.E.	ANA JULIA ESTUMANO MARTINS	2882
MARTINS, A.J.E.	ANA JULIA ESTUMANO MARTINS	3034
MARTINS, A.J.E.	ANA JULIA ESTUMANO MARTINS	3380
MARTINS, A.L.	ARIELI LEDUR MARTINS	3499
MARTINS, A.M.D.F.	ALESSANDRA MARCIA DA FONSECA MARTINS	3403
MARTINS, A.M.D.F.	ALESSANDRA MARCIA DA FONSECA MARTINS	2823

MARTINS, A.M.D.F.	ALESSANDRA MARCIA DA FONSECA MARTINS	2862
MARTINS, A.M.D.F.	ALESSANDRA MARCIA DA FONSECA MARTINS	2980
MARTINS, A.M.D.F.	ALESSANDRA MARCIA DA FONSECA MARTINS	3164
MARTINS, A.M.D.F.	ALESSANDRA MARCIA DA FONSECA MARTINS	3343
MARTINS, A.M.D.F.	ALESSANDRA MARCIA DA FONSECA MARTINS	3363
MARTINS, B.D.P.	BRUNA DE PAIVA MARTINS	2837
MARTINS, E.M.D.N.	ESTEFÂNIA MARA DO NASCIMENTO MARTINS	2674
MARTINS, F.D.S.	FLAVIANO DOS SANTOS MARTINS	2698
MARTINS, F.D.S.	FLAVIANO DOS SANTOS MARTINS	2735
MARTINS, I.M.	ISABELA MANCINI MARTINS	3351
MARTINS, J.R.	JENNIFFER RAMOS MARTINS	2686
MARTINS, J.R.	JENNIFFER RAMOS MARTINS	2988
MARTINS, L.C.	LETÍCIA CARDOSO MARTINS	2705
MARTINS, M.F.	MARTA FONSECA MARTINS	2906
MARTINS, M.F.	MARTA FONSECA MARTINS	3096
MARTINS, M.F.	MARTA FONSECA MARTINS	3450
MARTINS, N.S.	NÚBIA SABRINA MARTINS	3188
MARTINS, P.D.C.	PRISCILLA DA COSTA MARTINS	2940
MARTINS, P.F.D.S.	PRISCILA FERNANDA DA SILVA MARTINS	3294
MARTINS, P.M.R.E.S.	PATRÍCIA MACHADO RODRIGUES E SILVA MARTINS	3278
MARTINS, R.	RAFAELA MARTINS	2860
MARTINS, R.B.	RONALDO B. MARTINS	3369
MARTINS, R.B.	RONALDO BRAGANÇA MARTINS	3468
MARTINS, R.S.	ROBERTTA SILVA MARTINS	3110
MARTINS, S.D.F.	SARA DE FREITAS MARTINS	2880
MARTINS, S.D.F.	SARA DE FREITAS MARTINS	3186
MARTINS, T.V.	TIMNA VARELA MARTINS	3333
MARTINS, V.D.	VINICIUS DANTAS MARTINS	2673
MARTINS, V.D.	VINICIUS DANTAS MARTINS	2676
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2630
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2635
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2638
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2642
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2644
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2649
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2650
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2652
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2660
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	2715
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	3219
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	3294
MARTINS-FILHO, O.A.	OLINDO ASSIS MARTINS-FILHO	3395
MARTORANO, R.M.	RODRIGO MEDEIROS MARTORANO	2893
MARTORANO, R.M.	RODRIGO MEDEIROS MARTORANO	3050
MARTYRES, A.	ANDREZA MARTYRES	2797
MARTYRES, A.	ANDREZA MARTYRES	2995
MASCARENHAS, D.P.A.	DANIELLE PINI ALVES MASCARENHAS	3296
MASCARENHAS, D.P.A.	DANIELLE PINI ALVES MASCARENHAS	3367
MASCARENHAS, D.P.A.	DANIELLE PINI ALVES MASCARENHAS	3384
MASCARENHAS, G.D.	GLENDA DOMINGOS MASCARENHAS	2758
MASCARENHAS, G.D.	GLENDA DOMINGOS MASCARENHAS	3014
MASCARENHAS, G.D.	GLENDA DOMINGOS MASCARENHAS	3105
MASCARENHAS, G.D.	GLENDA DOMINGOS MASCARENHAS	3274
MASCARENHAS, G.D.	GLENDA DOMINGOS MASCARENHAS	3426
MASSARDI, C.D.	CAIO DIAS MASSARDI	2702
MASSET, M.E.G.	MARIA EDUARDA GLOBA MASSET	2825
MASSET, M.E.G.	MARIA EDUARDA GLOBA MASSET	2830
MASSET, M.E.G.	MARIA EDUARDA GLOBA MASSET	2859
MASSET, M.E.G.	MARIA EDUARDA GLOBA MASSET	2864

MASSET, M.E.G.	MARIA EDUARDA GLOBA MASSET	2881
MASSET, M.E.G.	MARIA EDUARDA GLOBA MASSET	2888
MASSET, M.E.G.	MARIA EDUARDA GLOBA MASSET	2901
MATA-SANTOS, H.A.	HILTON ANTÔNIO MATA-SANTOS	3070
MATAVEL, A.C.D.S.	ALESSANDRA CRISTINA DE SOUZA MATAVEL	2953
MATEUS, L.C.O.	LAIZA CAMILA OLIVEIRA MATEUS	3352
MATIAS, D.O.	DAIANE OLIVEIRA MATIAS	3065
MATIAS, D.O.	DAIANE OLIVEIRA MATIAS	3371
MATOS, A.D.S.	ADA DA SILVA MATOS	2743
MATSUDA, E.M.	ELAINE MONTEIRO MATSUDA	3002
MATSUMURA, E.L.M.	ELISA LIE MARTINES MATSUMURA	2855
MATSUMURA, E.L.M.	ELISA LIE MARTINES MATSUMURA	3236
MATTA, L.	LORENA MATTA	2903
MATTEUCCI, K.C.	KELY CATARINE MATTEUCCI	3337
MATTEUCCI, K.C.	KELY CATARINE MATTEUCCI	3513
MATTOS, G.H.R.	GUILHERME HENRIQUE RODRIGUES MATTOS	2989
MATTOS, G.H.R.	GUILHERME HENRIQUE RODRIGUES MATTOS	3008
MATTOS, G.H.R.	GUILHERME HENRIQUE RODRIGUES MATTOS	3230
MATTOS, G.H.R.	GUILHERME HENRIQUE RODRIGUES MATTOS	3325
MATTOS, T.R.F.	THAYANA ROBERTA FERREIRA MATTOS	3041
MATTOS, T.R.F.	THAYANA ROBERTA FERREIRA MATTOS	3071
MATTOS, T.R.F.	THAYANA ROBERTA FERREIRA MATTOS	3278
MAURO, E.F.D.L.	ELIANA FAQUIM DE LIMA MAURO	3218
MAURO, E.F.D.L.	ELIANA FAQUIM DE LIMA MAURO	3225
MAURO, E.F.D.L.	ELIANA FAQUIM DE LIMA MAURO	3277
MAXIMO, M.L.D.S.	MATHEUS LACERDA DE SOUZA MAXIMO	2915
MAZZINGHY, G.S.	GLEISLA SANTOS MAZZINGHY	2634
MAZZOCCO, Y.L.	YANINA LUCIANA MAZZOCCO	2840
MCAULIFFE, J.	JAMES MCAULIFFE	2929
MCCOY, K.	KATHY MCCOY	2926
MCCOY, K.	KATHY MCCOY	3449
MCDOWELL, J.	JOSEPH MCDOWELL	2844
MCGETTRICK, A.	ANNE MCGETTRICK	3487
MEDEIROS, E.B.	EDUARDA BEHENCK MEDEIROS	3118
MEDINA, T.	TIAGO MEDINA	3039
MEDINA, T.	TIAGO MEDINA	3392
MEDINA, T.D.S.	TIAGO DA SILVA MEDINA	2827
MEDINA, T.D.S.	TIAGO DA SILVA MEDINA	2841
MEDINA, T.D.S.	TIAGO DA SILVA MEDINA	3029
MEDINA, T.D.S.	TIAGO DA SILVA MEDINA	3094
MEDINA, T.D.S.	TIAGO DA SILVA MEDINA	3171
MEDINA, T.D.S.	TIAGO DA SILVA MEDINA	3309
MEDINA-ACOSTA, E.	ENRIQUE MEDINA-ACOSTA	2994
MEDINA-ACOSTA, E.	ENRIQUE MEDINA-ACOSTA	2996
MEDINA-ACOSTA, E.	ENRIQUE MEDINA-ACOSTA	3021
MEDINA-ACOSTA, E.	ENRIQUE MEDINA-ACOSTA	3035
MEGGIOLARO, E.D.A.	EMILLY DUTRA AMARAL MEGGIOLARO	3281
MEINEL, R.S.	RAISSA SOARES MEINEL	3082
MEINEL, R.S.	RAISSA SOARES MEINEL	3102
MEINEL, R.S.	RAISSA SOARES MEINEL	3147
MEINEL, R.S.	RAISSA SOARES MEINEL	3162
MEINEL, R.S.	RAISSA SOARES MEINEL	3203
MEIRA, A.C.M.	ANA CLARA MARTINS MEIRA	2651
MEIRA, R.D.O.	RAQUEL DE OLIVEIRA MEIRA	2681
MELENDEZ, M.E.	MATIAS ELISEO MELENDEZ	3038
MELGAÇO, J.G.	JULIANA GIL MELGAÇO	2812
MELGAÇO, J.G.	JULIANA GIL MELGAÇO	3154
MELGAÇO, J.G.	JULIANA GIL MELGAÇO	3243
MELGAÇO, J.G.	JULIANA GIL MELGAÇO	3411

MELO, G.A.	GUILHERME AFONSO MELO	3150
MELO, G.A.	GUILHERME AFONSO MELO	3394
MELO, L.D.	LÉON DIMITRI MELO	2775
MELO, L.D.S.	LUCAS DOS SANTOS MELO	2790
MELO, N.	NATÁLIA MELO	2651
MELO, N.	NATÁLIA MELO	2698
MELO, N.	NATÁLIA MELO	2701
MELO, N.	NATÁLIA MELO	3470
MELO, V.C.	VICTOR C. MELO	2763
MELO, V.C.	VICTOR C. MELO	2764
MELO, V.L.M.	VÍTOR LIMA MIRANDA MELO	3182
MELTZER, R.	REBECCA MELTZER	2882
MEMÓRIA, K.A.S.	KATHELEN ANNE SUDO MEMÓRIA	3414
MENDES, A.S.	ATLANTE S. MENDES	2852
MENDES, C.M.	CAROLINA MAIA MENDES	2897
MENDES, C.M.	CAROLINA MAIA MENDES	3099
MENDES, C.M.	CAROLINA MAIA MENDES	3132
MENDES, C.M.	CAROLINA MAIA MENDES	3138
MENDES, C.M.	CAROLINA MAIA MENDES	3195
MENDES, C.M.	CAROLINA MAIA MENDES	3238
MENDES, C.M.	CAROLINA MAIA MENDES	3241
MENDES, C.M.	CAROLINA MAIA MENDES	3256
MENDES, C.M.	CAROLINA MAIA MENDES	3261
MENDES, E.	ELIZABETH MENDES	3358
MENDES, G.E.R.	GABRIEL EDUARDO RIBEIRO MENDES	2687
MENDES, G.E.R.	GABRIEL EDUARDO RIBEIRO MENDES	2690
MENDES, M.J.	MARIANE JULIELE MENDES	2652
MENDES, M.T.	MARIA TAYS MENDES	3158
MENDES, M.T.	MARIA TAYS MENDES	3372
MENDES, N.S.	NATALIA SERRA MENDES	2875
MENDES-AGUIAR, C.D.O.	CAROLINA DE OLIVEIRA MENDES-AGUIAR	3068
MENDES-DA-CRUZ, D.A.	DANIELLA AREAS MENDES-DA-CRUZ	3052
MENDES-DA-CRUZ, D.A.	DANIELLA AREAS MENDES-DA-CRUZ	3057
MENDES-DE-ALMEIDA, D.P.	DANIELA P. MENDES-DE-ALMEIDA	3184
MENDES-DE-ALMEIDA, D.P.	DANIELA P. MENDES-DE-ALMEIDA	3249
MENDES-DE-ALMEIDA, D.P.	DANIELA P. MENDES-DE-ALMEIDA	3411
MENDONÇA, G.D.S.	GUSTAVO DE SOUZA MENDONÇA	3126
MENDONÇA, H.R.	HENRIQUE ROCHA MENDONÇA	3110
MENEGATI, L.M.	LAURA MACHADO MENEGATI	2801
MENEGATI, L.M.	LAURA MACHADO MENEGATI	2813
MENEGATI, L.M.	LAURA MACHADO MENEGATI	2822
MENEGATI, L.M.	LAURA MACHADO MENEGATI	3116
MENEZES, G.	GUSTAVO MENEZES	3305
MENEZES, G.	GUSTAVO MENEZES	3334
MENEZES, G.	GUSTAVO MENEZES	3433
MENEZES, G.	GUSTAVO MENEZES	3465
MENEZES-SILVA, L.	LUÍSA MENEZES-SILVA	2944
MERCURI, R.L.V.	RAFAEL LUIZ VIEIRA MERCURI	3126
MESCHICK, C.G.	CARLOS GUILHERME MESCHICK	3054
MESSIAS, I.M.	IARA MONIQUE MESSIAS	3060
MESSIAS, I.M.	IARA MONIQUE MESSIAS	3314
MESSIAS, I.M.	IARA MONIQUE MESSIAS	3364
MESSNER, F.	FABIANE MESSNER	3295
MEYER, R.	ROBERTO MEYER	3282
MEYER, R.	ROBERTO MEYER	3373
MEYER, R.	ROBERTO MEYER	3406
MEYER, R.	ROBERTO MEYER	3441
MEYER, R.	ROBERTO MEYER	3504
MEYRELLES, M.S.M.	MAYRA S MACHADO MEYRELLES	2912

MIGUEL, D.C.	DANILO CICCONE MIGUEL	3034
MILANEZI, C.M.	CRISTIANE MARIA MILANEZI	2875
MILESI, L.M.	LUANA MEICHTRY MILESI	3117
MILESI, L.M.	LUANA MEICHTRY MILESI	3368
MILL, J.G.	JOSE GERALDO MILL	3059
MIN, Y.	YUKUN MIN	3487
MINATI, L.S.	LAIS STEPHANIE MINATI	2884
MINATI, L.S.	LAIS STEPHANIE MINATI	3056
MINEO, J.R.	JOSÉ ROBERTO MINEO	2689
MINEO, T.W.P.	TIAGO WILSON PATRIARCA MINEO	2689
MINEO, T.W.P.	TIAGO WILSON PATRIARCA MINEO	2704
MINEO, T.W.P.	TIAGO WILSON PATRIARCA MINEO	2706
MINEO, T.W.P.	TIAGO WILSON PATRIARCA MINEO	2707
MINER, J.J.	JONATHAN J. MINER	3506
MIRANDA, C.B.S.	CARLA BEATRIZ SANTOS MIRANDA	3370
MIRANDA, G.O.	GIULIA OLIVEIRA MIRANDA	3231
MIRANDA, G.P.	GABRIEL PASCHOAL MIRANDA	3237
MIRANDA, L.V.D.J.	LETÍCIA VICTÓRIA DE JESUS MIRANDA	3165
MIRANDA, V.D.S.	VANESSA DOS SANTOS MIRANDA	2689
MIRANDA, V.H.S.	VITOR HUGO SIMÕES MIRANDA	2667
MITSUGI, T.G.	THIAGO GIOVE MITSUGI	3472
MOLENTO, C.F.M.	CARLA FORTE MAIOLINO MOLENTO	3262
MOLINA, D.A.	DENIS ALEXIS MOLINA	2672
MOLINA, F.	FRANCK MOLINA	2683
MONATH, T.	THOMAS MONATH	2644
MONATH, T.	THOMAS MONATH	2652
MONTAÑA, J.E.L.	JHON EMERSON LOZANO MONTAÑA	3100
MONTEIRO, A.C.	ANA CAROLINA MONTEIRO	2825
MONTEIRO, A.C.	ANA CAROLINA MONTEIRO	2830
MONTEIRO, A.C.	ANA CAROLINA MONTEIRO	2859
MONTEIRO, A.C.	ANA CAROLINA MONTEIRO	2864
MONTEIRO, A.C.	ANA CAROLINA MONTEIRO	2881
MONTEIRO, A.C.	ANA CAROLINA MONTEIRO	2888
MONTEIRO, A.C.	ANA CAROLINA MONTEIRO	2901
MONTEIRO, D.	DAISY MONTEIRO	3219
MONTEIRO, F.B.F.	FABIOLA BRANCO FILIPPIN MONTEIRO	3300
MONTEIRO, G.M.	GIULIANNA MERRELHO MONTEIRO	2795
MONTEIRO, G.R.C.	GRAZIELE RODRIGUEZ CARLOS MONTEIRO	3333
MONTEIRO, L.D.B.	LAUAR DE BRITO MONTEIRO	2926
MONTEIRO, M.C.	MARTA CHAGAS MONTEIRO	3408
MONTEIRO, M.C.	MARTA CHAGAS MONTEIRO	3522
MONTEIRO, R.Q.	ROBSON Q. MONTEIRO	3329
MONTEIRO, V.V.S.	VALTER VINICIUS SILVA MONTEIRO	2719
MORAES, E.C.D.S.	EMILLY CAROLINE DOS SANTOS MORAES	3329
MORAES, J.D.S.	JÚLIA DOS SANTOS MORAES	3395
MORAES, S.K.	SABRINA KRICE MORAES	3454
MORAES, S.K.	SABRINA KRICE MORAES	3486
MORAES, S.K.	SABRINA KRICE MORAES	3493
MORAES, S.K.	SABRINA KRICE MORAES	3495
MORAES, S.K.	SABRINA KRICE MORAES	3497
MORAES, S.K.	SABRINA KRICE MORAES	3516
MORAES, T.D.F.S.	THAÍS DE FÁTIMA SILVA MORAES	2674
MORAES, T.D.F.S.	THAÍS DE FÁTIMA SILVA MORAES	3251
MORAIS, K.L.P.	KATIA LUCIANO PEREIRA MORAIS	2842
MORAIS, K.L.P.	KATIA LUCIANO PEREIRA MORAIS	2976
MORAIS, K.L.P.	KATIA LUCIANO PEREIRA MORAIS	3060
MORAIS, K.L.P.	KATIA LUCIANO PEREIRA MORAIS	3134
MORAIS, K.L.P.	KATIA LUCIANO PEREIRA MORAIS	3293
MORAIS, K.L.P.	KATIA LUCIANO PEREIRA MORAIS	3314

MORAIS, K.L.P.	KATIA LUCIANO PEREIRA MORAIS	3364
MOREIRA JUNIOR, R.E.	RENATO ELIAS MOREIRA JUNIOR	2684
MOREIRA JUNIOR, R.E.	RENTAO ELIAS MOREIRA JUNIOR	2676
MOREIRA, B.L.C.	BEATRIZ LOURENCO CORREIA MOREIRA	2857
MOREIRA, F.	FRANCIELLY MOREIRA	2944
MOREIRA, F.C.	FELIPE CAIXETA MOREIRA	2643
MOREIRA, F.C.	FELIPE CAIXETA MOREIRA	2645
MOREIRA, F.C.	FELIPE CAIXETA MOREIRA	2647
MOREIRA, F.C.	FELIPE CAIXETA MOREIRA	2664
MOREIRA, F.C.	FELIPE CAIXETA MOREIRA	2678
MOREIRA, F.C.	FELIPE CAIXETA MOREIRA	2682
MOREIRA, F.C.	FELIPE CAIXETA MOREIRA	2684
MOREIRA, F.C.	FELIPE CAIXETA MOREIRA	3067
MOREIRA, F.D.A.	FABRICIO DE ARAUJO MOREIRA	2847
MOREIRA, F.D.P.P.	FERNANDA DE PAULA PEPINO MOREIRA	2912
MOREIRA, F.D.P.P.	FERNANDA DE PAULA PEPINO MOREIRA	2936
MOREIRA, F.D.P.P.	FERNANDA DE PAULA PEPINO MOREIRA	2948
MOREIRA, F.M.	FRANCISCO MÔNICO MOREIRA	2736
MOREIRA, G.L.D.S.	GABRIELA LOUISE DA SILVA MOREIRA	3311
MOREIRA, I.B.G.	ISABELA BATISTA GONÇALVES MOREIRA	3426
MOREIRA, I.B.G.	ISABELA BATISTA GONÇALVES MOREIRA	3480
MOREIRA, I.Z.	ISABELLA ZAIDAN MOREIRA	2808
MOREIRA, I.Z.	ISABELLA ZAIDAN MOREIRA	3271
MOREIRA, I.Z.	ISABELLA ZAIDAN MOREIRA	3367
MOREIRA, I.Z.	ISABELLA ZAIDAN MOREIRA	3492
MOREIRA, L.D.S.	LETÍCIA DOS SANTOS MOREIRA	3100
MOREIRA, L.O.	LILIAN OLIVEIRA MOREIRA	2968
MOREIRA, L.O.	LILIAN OLIVEIRA MOREIRA	3090
MOREIRA, L.O.	LILIAN OLIVEIRA MOREIRA	3095
MOREIRA, M.	MILENE MOREIRA	2717
MOREIRA, M.D.B.P.	MÁRCIA DE BERREDO PINHO MOREIRA	3228
MOREIRA, M.D.B.P.	MÁRCIA DE BERREDO PINHO MOREIRA	3237
MOREIRA, M.D.B.P.	MÁRCIA DE BERREDO PINHO MOREIRA	3466
MOREIRA, M.S.	MELL SOUZA MOREIRA	2712
MOREIRA, P.O.L.	PAULO OTÁVIO LOURENÇO MOREIRA	2779
MOREIRA, T.B.	THAYSE BATISTA MOREIRA	2790
MOREIRA, T.B.	THAYSE BATISTA MOREIRA	3026
MOREIRA, T.B.	THAYSE BATISTA MOREIRA	3028
MOREIRA-NASCIMENTO, S.L.	SÂMICK L. MOREIRA-NASCIMENTO	2666
MORENO, A.C.R.	ANA CAROLINA RAMOS MORENO	2761
MORENO, A.C.R.	ANA CAROLINA RAMOS MORENO	2771
MORENO, A.C.R.	ANA CAROLINA RAMOS MORENO	2791
MORENO, A.C.R.	ANA CAROLINA RAMOS MORENO	2793
MORENO, M.	MARIA MORENO	2951
MORETTI, E.H.	EDUARDO H MORETTI	3416
MORETTI, N.S.	NILMAR SILVIO MORETTI	2779
MORGADO, E.S.	ELISSA SANTOS MORGADO	3174
MORGADO, E.S.	ELISSA SANTOS MORGADO	3268
MORGADO, M.G.	MARIZA G. MORGADO	3000
MORGADO, M.G.	MARIZA G. MORGADO	3064
MORGADO, M.G.	MARIZA G. MORGADO	3240
MORGADO, M.G.	MARIZA G. MORGADO	3260
MORGADO, M.G.	MARIZA GONÇALVES MORGADO	3105
MORGAN, H.	HENRIQUE MORGAN	3269
MORI, G.G.	GRAZIELA GARRIDO MORI	2736
MORI, G.G.	GRAZIELA GARRIDO MORI	2749
MORI, G.G.	GRAZIELA GARRIDO MORI	2934
MORI, M.A.D.S.	MARCELO ALVES DA SILVA MORI	2920
MORI, M.A.D.S.	MARCELO ALVES DA SILVA MORI	3111

MORROT, A.	ALEXANDRE MORROT	2738
MORROT, A.	ALEXANDRE MORROT	3232
MORROT, A.	ALEXANDRE MORROT	3484
MOSIMANN, A.L.P.	ANA LUIZA PAMPLONA MOSIMANN	2857
MOSIMANN, A.L.P.	ANA LUIZA PAMPLONA MOSIMANN	2916
MOSSER, D.	DAVID MOSSER	2862
MOSSER, D.	DAVID MOSSER	2868
MOTA, C.D.L.	CAROLINE DE LIMA MOTA	3471
MOTA, C.D.L.	CAROLINE DE LIMA MOTA	3501
MOTA, C.M.	CAROLINE MARTINS MOTA	2704
MOTA, C.M.	CAROLINE MARTINS MOTA	2707
MOTA, R.A.	RINALDO APARECIDO MOTA	3010
MOTA, S.D.J.	SILVIA DE JESUS MOTA	2769
MOTA, S.D.J.	SILVIA DE JESUS MOTA	2776
MOTTA, J.M.	JULIANA MARIA MOTTA	3070
MOTTRAN, J.C.	JEREMY C. MOTTRAN	3276
MOURA, F.	FERNANDO MOURA	3060
MOURA, L.V.	LEONARDO VICENTE MOURA	2770
MOYSES, M.N.	MAURICIO NOGUEIRA MOYSES	3326
MUCIDA, D.	DANIEL MUCIDA	2798
MUEHLMANN, L.A.	LUIS A. MUEHLMANN	2763
MUHLEN, P.H.F.V.	PEDRO HENRIQUE FILIPIN VON MUHLEN	3289
MUHLEN, P.H.F.V.	PEDRO HENRIQUE FILIPIN VON MUHLEN	3353
MUKHI, D.	DHANUNJAY MUKHI	3506
MULLER, R.	RODRIGO MULLER	2812
MUNHOZ, C.D.	CAROLINA DEMARCHI MUNHOZ	3230
MUNIZ, L.M.C.	LUANA MEL COUTINHO MUNIZ	3090
MUNIZ, M.F.R.	MARIA FERNANDA RODRIGUES MUNIZ	3501
MUNIZ, V.D.S.	VALDIRENE DE SOUZA MUNIZ	3328
MUÑOZ, V.R.	VITOR ROSETTO MUÑOZ	2869
MURRAY, P.J.	PETER JONATHAN MURRAY	3004
MURTA, G.K.T.	GABRIELA KALIFF TEÓFILO MURTA	2671
MURTA, G.K.T.	GABRIELA KALIFF TEÓFILO MURTA	2681
MURTA, G.K.T.	GABRIELA KALIFF TEÓFILO MURTA	2688
MUTARELLI, A.	ANTÔNIO MUTARELLI	2976
MUXEL, S.M.	SANDRA MARIA MUXEL	2889
NAGIB, P.R.A.	PATRÍCIA RESENDE ALO NAGIB	2979
NAGIB, P.R.A.	PATRÍCIA RESENDE ALO NAGIB	3342
NAHN JUNIOR, E.P.	EDILBERT PELLEGRINI NAHN JUNIOR	3377
NAHN JUNIOR, E.P.	EDILBERT PELLEGRINI NAHN JUNIOR	3405
NAIK, S.	SHRUTI NAIK	3244
NAKAMA, R.P.	RAQUEL PIRES NAKAMA	2729
NAKAMA, R.P.	RAQUEL PIRES NAKAMA	3104
NAKAMA, R.P.	RAQUEL PIRES NAKAMA	3180
NAKAMA, R.P.	RAQUEL PIRES NAKAMA	3438
NAKANDAKARI, S.C.B.R.	SUSANA CASTELO BRANCO RAMOS NAKANDAKARI	2869
NAKAYA, H.I.	HELDER IMOTO NAKAYA	2647
NAKAYA, H.I.	HELDER IMOTO NAKAYA	2882
NAKAYA, H.I.	HELDER IMOTO NAKAYA	2935
NAKHASI, H.L.	HIRA L NAKHASI	3210
NARDELLI, S.C.	SHEILA CRISTINA NARDELLI	2916
NARDINI, V.	VIVIANI NARDINI	3344
NASCIMENTO, A.L.R.	ANA LUCIA ROSA NASCIMENTO	3055
NASCIMENTO, C.D.M.C.	CAMILA DE MELO CARVALHO NASCIMENTO	2816
NASCIMENTO, C.D.M.C.	CAMILA DE MELO CARVALHO NASCIMENTO	2857
NASCIMENTO, C.D.M.C.	CAMILA DE MELO CARVALHO NASCIMENTO	2995
NASCIMENTO, C.D.M.C.	CAMILA DE MELO CARVALHO NASCIMENTO	3006
NASCIMENTO, C.R.	CAMYLE RODRIGUES NASCIMENTO	3013
NASCIMENTO, C.R.	CAMYLE RODRIGUES NASCIMENTO	3299

NASCIMENTO, C.R.	CAMYLE RODRIGUES NASCIMENTO	3301
NASCIMENTO, C.S.	CAMILA SALES NASCIMENTO	2653
NASCIMENTO, C.S.	CAMILA SALES NASCIMENTO	2654
NASCIMENTO, D.D.O.	DANIELLE DE OLIVEIRA NASCIMENTO	2918
NASCIMENTO, D.D.O.	DANIELLE DE OLIVEIRA NASCIMENTO	3427
NASCIMENTO, F.C.	FREDERICO C. NASCIMENTO	2655
NASCIMENTO, I.A.	ISABELLE ANTUNES NASCIMENTO	2918
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	2897
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	2904
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	3099
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	3132
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	3138
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	3195
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	3238
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	3241
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	3256
NASCIMENTO, J.G.	JULIANA GUEDES NASCIMENTO	3261
NASCIMENTO, L.S.	LETÍCIA SILVA NASCIMENTO	3377
NASCIMENTO, M.F.A.	MARIA F. A. NASCIMENTO	2666
NASCIMENTO, M.T.	MAURÍCIO TEIXEIRA NASCIMENTO	3098
NASTARO, P.	PEDRO NASTARO	3335
NAVARRO, A.S.	ANDREA SANCHEZ NAVARRO	3506
NAVEGA, A.V.	ALEXIA VITÓRIA NAVEGA	3463
NAVEGANTES, L.C.	LUIZ CARLOS NAVEGANTES	3269
NAZIOBERTO DUDA DE FARIAS, J.	JOSÉ NAZIOBERTO DUDA DE FARIAS	2795
NAZIOBERTO DUDA DE FARIAS, J.	JOSÉ NAZIOBERTO DUDA DE FARIAS	3086
NAZIOBERTO DUDA DE FARIAS, J.	JOSÉ NAZIOBERTO DUDA DE FARIAS	3166
NAZZARI, A.	ALEXANDRA NAZZARI	2789
NEGREIROS, N.G.S.	NÁTHANI GABRIELLY SILVA NEGREIROS	2722
NERES, P.C.C.	PRISCILA CRISTINA CORRÊA NERES	3129
NERY, B.	BRENDA NERY	2941
NERY, B.	BRENDA NERY	2977
NERY-NETO, J.A.D.O.	JOSÉ ARIMATÉA DE OLIVEIRA NERY-NETO	2909
NERY-NETO, J.A.D.O.	JOSÉ ARIMATÉA DE OLIVEIRA NERY-NETO	2917
NETO, A.C.D.O.	ANTÔNIO CLEMENTE DE OLIVEIRA NETO	3375
NETO, A.D.	AMARO DUARTE NETO	2792
NETO, A.F.	ADOLFO FIRMINO NETO	2834
NETO, D.T.D.S.	DAYSE TEIXEIRA DA SILVA NETO	3311
NETO, E.D.A.	EURICO DE ARRUDA NETO	3468
NETO, F.M.D.S.	FIRMINO MARTINS DOS SANTOS NETO	3303
NETO, F.M.D.S.	FIRMINO MARTINS DOS SANTOS NETO	3307
NETO, F.M.D.S.	FIRMINO MARTINS DOS SANTOS NETO	3381
NETO, F.P.D.S.	FRANCISCO PORFIRIO DA SILVA NETO	3448
NETO, H.A.D.P.	HEITOR AFFONSO DE PAULA NETO	3150
NETO, H.A.D.P.	HEITOR AFFONSO DE PAULA NETO	3295
NETO, H.A.D.P.	HEITOR AFFONSO DE PAULA NETO	3433
NETO, H.C.D.C.F.	HUGO CAIRE DE CASTRO FARIA NETO	3329
NETO, J.A.C.	JUNIEL ASSIS CRESPO NETO	3383
NETO, J.A.C.	JUNIEL ASSIS CRESPO NETO	3414
NETO, J.B.	JOSÉ BARCELLO NETO	3079
NETO, J.B.	JOSÉ BARCELLO NETO	3264
NETO, J.B.	JOSE B NETO	3219
NETO, J.D.A.	JOSÉ DJACI AUGUSTO NETO	3388
NETO, J.P.D.	JÃO PEREIRA DUPRAT NETO	3309
NETO, M.A.N.	MOISÉS AGUIAR NEVES NETO	3150
NETO, M.A.N.	MOISÉS AGUIAR NEVES NETO	3394
NETO, M.B.	MANOEL BARRAL NETO	2991
NETO, P.P.D.O.	PERGENTINO POSSIDÔNIO DE OLIVEIRA NETO	3432
NETO, P.V.D.S.	PEDRO VIEIRA DA SILVA NETO	3303

NETO, P.V.D.S.	PEDRO VIEIRA DA SILVA NETO	3381
NETO, R.L.D.M.	RUBENS LIMA DO MONTE NETO	2779
NEUMANN, A.D.S.	ARTHUR DA SILVA NEUMANN	3113
NEUMANN, A.D.S.	ARTHUR DA SILVA NEUMANN	3142
NEUMANN, A.D.S.	ARTHUR DA SILVA NEUMANN	3153
NEVES, A.V.B.	ANA VICTORIA BARBOSA NEVES	2635
NEVES, C.L.	CAMILA LIMA NEVES	2902
NEVES, E.G.A.	EULA GRACIELE AMORIM NEVES	2790
NEVES, E.G.A.	EULA GRACIELE AMORIM NEVES	3028
NEVES, E.G.A.	EULA GRACIELE AMORIM NEVES	3234
NEVES, J.C.F.	JULIANA COSTA FERREIRA NEVES	3395
NEVES, L.D.O.F.	LAURA DE OLIVEIRA FARIAS NEVES	2919
NEVES, P.C.D.C.	PATRÍCIA C. DA COSTA NEVES	2743
NEVES, P.C.D.C.	PATRÍCIA C. DA COSTA NEVES	2778
NEVES, P.C.D.C.	PATRÍCIA C. DA COSTA NEVES	2812
NEVES, P.C.D.C.	PATRÍCIA C. DA COSTA NEVES	3154
NEVES, P.C.D.C.	PATRÍCIA C. DA COSTA NEVES	3159
NIELSEN, M.	MORTEN NIELSEN	3033
NILSSON, J.B.	JONAS BIRKELUND NILSSON	3033
NIMRICHTER, L.	LEONARDO NIMRICHTER	2815
NIMRICHTER, L.	LEONARDO NIMRICHTER	3090
NIMRICHTER, L.	LEONARDO NIMRICHTER	3484
NIRELLO, V.D.	VINÍCIUS DIAS NIRELLO	3067
NIRELLO, V.D.	VINÍCIUS DIAS NIRELLO	3220
NISIMURA, L.M.	LINDICE MITIE NISIMURA	2857
NÓBEREGA, A.	ALBERTO NÓBEREGA	3068
NOBLECOURT, L.	LAURINE NOBLECOURT	2929
NÓBREGA, A.	ALBERTO NÓBREGA	2724
NÓBREGA, A.	ALBERTO NÓBREGA	2888
NÓBREGA, A.	ALBERTO NÓBREGA	3178
NÓBREGA, I.B.C.	ISABELA BEATRIZ CABACINHA NÓBREGA	2656
NÓBREGA, I.B.C.	ISABELA BEATRIZ CABACINHA NÓBREGA	2684
NODARI, J.Z.	JOANA ZORZAL NODARI	3409
NOGUEIRA, A.D.R.	ALANDERSON DA ROCHA NOGUEIRA	3311
NOGUEIRA, A.L.	ANA L. NOGUEIRA	2833
NOGUEIRA, F.C.S.	FÁBIO CÉSAR SOUSA NOGUEIRA	2810
NOGUEIRA, H.A.C.	HIGOR ALMEIDA CORDEIRO NOGUEIRA	2994
NOGUEIRA, H.A.C.	HIGOR ALMEIDA CORDEIRO NOGUEIRA	2996
NOGUEIRA, H.A.C.	HIGOR ALMEIDA CORDEIRO NOGUEIRA	3021
NOGUEIRA, H.A.C.	HIGOR ALMEIDA CORDEIRO NOGUEIRA	3035
NOGUEIRA, J.N.	JÚLIA NUNES NOGUEIRA	3358
NOGUEIRA, M.L.	MAURÍCIO LACERDA NOGUEIRA	3294
NOGUEIRA, P.A.	PAULO AFONSO NOGUEIRA	3083
NOGUEIRA, P.A.	PAULO AFONSO NOGUEIRA	3272
NOGUEIRA, P.M.	PAULA MONALISA NOGUEIRA	2779
NOGUEIRA, R.S.	ROBERTA SCHIAVON NOGUEIRA	3362
NOGUEIRA, S.M.	SIMONE MITRI NOGUEIRA	3501
NOGUEIRA, T.D.O.	THAIS DE OLIVEIRA NOGUEIRA	2728
NOGUEIRA7, L.P.	LIEBERT PARREIRAS NOGUEIRA7	2901
NONATO, A.	ANDERSON NONATO	2698
NONATO, A.	ANDERSON NONATO	2701
NONATO, A.	ANDERSON NONATO	2702
NONATO, O.C.D.S.	OSVALDO CAMPOS DOS SANTOS NONATO	3376
NONATO, O.C.D.S.	OSVALDO CAMPOS DOS SANTOS NONATO	3428
NORLING, L.	LUCY NORLING	3329
NORONHA, A.	ANDRESSA NORONHA	2972
NORTON, E.	ELIZABETH NORTON	2734
NOVAES, R.D.	RÔMULO DIAS NOVAES	2962
NOVAES, S.D.	SÂMEK DANTAS NOVAES	2871

NOVAES, S.D.	SÂMEK DANTAS NOVAES	2887
NOVAES, S.D.	SÂMEK DANTAS NOVAES	2910
NOVAES, S.D.	SÂMEK DANTAS NOVAES	3025
NTUMNGIA, F.B.	FRANCIS B. NTUMNGIA	2666
NUNES, A.D.S.	ANDERSON DE SÁ NUNES	2811
NUNES, A.D.S.	ANDERSON DE SÁ NUNES	3416
NUNES, C.M.D.C.	CAMILA MARSANO DA COSTA NUNES	3293
NUNES, C.M.D.C.	CAMILA MARSANO DA COSTA NUNES	3314
NUNES, C.M.D.C.	CAMILA MARSANO DA COSTA NUNES	3364
NUNES, C.P.	CLAUDIA PAIVA NUNES	3031
NUNES, C.P.	CLAUDIA PAIVA NUNES	3289
NUNES, C.P.	CLAUDIA PAIVA NUNES	3353
NUNES, C.P.	CLAUDIA PAIVA NUNES	3488
NUNES, G.C.	GLAUCIA COLETTI NUNES	3119
NUNES, J.B.	JULIANA BARBOSA NUNES	3246
NUNES, J.E.S.	JOSÉ EDUARDO SACCONI NUNES	3023
NUNES, J.E.S.	JOSÉ EDUARDO SACCONI NUNES	3031
NUNES, J.P.D.S.	JOÃO PEDRO DA SILVA NUNES	2740
NUNES, J.P.D.S.	JOÃO PEDRO DA SILVA NUNES	3211
NUNES, J.P.S.	JOÃO PAULO SILVA NUNES	3074
NUNES, M.D.C.P.	MARIA DO CARMO PEREIRA NUNES	2790
NUNES, M.D.C.P.	MARIA DO CARMO PEREIRA NUNES	2972
NUNES, M.D.C.P.	MARIA DO CARMO PEREIRA NUNES	2976
NUNES, M.D.C.P.	MARIA DO CARMO PEREIRA NUNES	3026
NUNES, M.D.C.P.	MARIA DO CARMO PEREIRA NUNES	3028
NUNES, M.D.C.P.	MARIA DO CARMO PEREIRA NUNES	3234
NUSSENZWEIG, M.C.	MICHEL C. NUSSENZWEIG	3007
O'CARROL, S.	SHANE O'CARROL	3487
OGONCZYK, D.	DANIELLA OGONCZYK	2780
OHASHI, P.	PAMELA OHASHI	2770
OJEYINKA, D.I.	DORCAS IDOWU OJEYINKA	3050
OKINO, C.H.	CINTIA HIROMI OKINO	2782
OLEAN-OLIVEIRA, T.	TIAGO OLEAN-OLIVEIRA	2866
OLEGGINI, A.	ANALÍA OLEGGINI	2820
OLIMPIO, F.	FABIANA OLIMPIO	2939
OLIVEIRA, A.C.D.	ANA CLÁUDIA DUMONT OLIVEIRA	3281
OLIVEIRA, A.D.N.	ANNAMAIRLLA DO NASCIMENTO OLIVEIRA	3453
OLIVEIRA, A.V.C.	ALYSSON VINÍCIUS COSTA OLIVEIRA	2718
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2673
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2734
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2735
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2741
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2878
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2889
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2938
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2944
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2966
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2967
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	2982
OLIVEIRA, B.D.C.	BERNARDO DE CASTRO OLIVEIRA	3076
OLIVEIRA, C.J.F.	CARLO JOSÉ FREIRE OLIVEIRA	3497
OLIVEIRA, D.M.	DOUGLAS MATHIAS OLIVEIRA	3490
OLIVEIRA, E.A.	ERIKA APARECIDA OLIVEIRA	3265
OLIVEIRA, E.L.D.S.	EVELLYN LORRAINE DE SOUSA OLIVEIRA	3398
OLIVEIRA, E.R.	EMILIANO ROSA OLIVEIRA	2658
OLIVEIRA, F.	FABIANO OLIVEIRA	3071
OLIVEIRA, F.B.R.	FERNANDO BENTO RODRIGUES OLIVEIRA	2698
OLIVEIRA, F.B.R.	FERNANDO BENTO RODRIGUES OLIVEIRA	2699
OLIVEIRA, F.B.R.	FERNANDO BENTO RODRIGUES OLIVEIRA	2701

OLIVEIRA, F.B.R.	FERNANDO BENTO RODRIGUES OLIVEIRA	2702
OLIVEIRA, F.D.S.	FELIPPE DOS SANTOS OLIVEIRA	3329
OLIVEIRA, G.D.N.	GUSTAVO DO NASCIMENTO OLIVEIRA	3071
OLIVEIRA, G.D.S.M.D.	GUILHERME DOS SANTOS MARBACK D' OLIVEIRA	3346
OLIVEIRA, G.M.	GIULLIANA MENEGOTTO OLIVEIRA	3186
OLIVEIRA, G.S.	GIOVANNA SANTOS OLIVEIRA	3002
OLIVEIRA, H.	HORTÊNCIA OLIVEIRA	3334
OLIVEIRA, H.M.D.C.	HORTÊNCIA MACIEL DE CASTRO OLIVEIRA	3305
OLIVEIRA, I.	ISADORA OLIVEIRA	3173
OLIVEIRA, J.D.S.B.	JULIANA DA SILVA BRANDI OLIVEIRA	3474
OLIVEIRA, J.E.	JEFFERSON ELIAS OLIVEIRA	2729
OLIVEIRA, J.E.	JEFFERSON ELIAS OLIVEIRA	2766
OLIVEIRA, J.E.	JEFFERSON ELIAS OLIVEIRA	3104
OLIVEIRA, J.E.	JEFFERSON ELIAS OLIVEIRA	3180
OLIVEIRA, J.E.	JEFFERSON ELIAS OLIVEIRA	3438
OLIVEIRA, J.F.	JAMILLE FERNANDES OLIVEIRA	3328
OLIVEIRA, J.K.A.	JÉSSICA KAROLINE AUGUSTA OLIVEIRA	2663
OLIVEIRA, J.P.L.	JOÃO PEDRO L. OLIVEIRA	3150
OLIVEIRA, K.C.	KAREN CRISTINA OLIVEIRA	3189
OLIVEIRA, K.C.	KAREN CRISTINA OLIVEIRA	3258
OLIVEIRA, K.C.	KAREN CRISTINA OLIVEIRA	3283
OLIVEIRA, K.C.	KAREN CRISTINA OLIVEIRA	3459
OLIVEIRA, K.C.	KAREN CRISTINA OLIVEIRA	3460
OLIVEIRA, L.A.R.	LAURA ALVES RIBEIRO OLIVEIRA	2705
OLIVEIRA, L.A.R.	LAURA ALVES RIBEIRO OLIVEIRA	2756
OLIVEIRA, L.A.R.	LAURA ALVES RIBEIRO OLIVEIRA	3267
OLIVEIRA, L.A.R.	LAURA ALVES RIBEIRO OLIVEIRA	3294
OLIVEIRA, L.B.P.	LORENA BUNA P. OLIVEIRA	3219
OLIVEIRA, L.P.G.	LUCAS PIRES GARCIA OLIVEIRA	2777
OLIVEIRA, M.D.A.	MARIANA DE ALMEIDA OLIVEIRA	2684
OLIVEIRA, M.S.	MATHEUS SILVA OLIVEIRA	2676
OLIVEIRA, N.F.D.M.	NATÁLIA FERNANDA DE MELO OLIVEIRA	2699
OLIVEIRA, N.F.D.M.	NATÁLIA FERNANDA DE MELO OLIVEIRA	2702
OLIVEIRA, P.D.M.	PATRÍCIA DE MELO OLIVEIRA	2692
OLIVEIRA, P.J.	PAULO J. OLIVEIRA	2866
OLIVEIRA, P.R.S.	PABLO RAFAEL SILVEIRA OLIVEIRA	3025
OLIVEIRA, P.R.S.	PABLO R S OLIVEIRA	2833
OLIVEIRA, P.R.S.	PABLO R S OLIVEIRA	2871
OLIVEIRA, P.R.S.	PABLO R S OLIVEIRA	2887
OLIVEIRA, P.R.S.	PABLO R S OLIVEIRA	2910
OLIVEIRA, P.R.S.	PABLO R S OLIVEIRA	3156
OLIVEIRA, P.R.S.	PABLO R S OLIVEIRA	3520
OLIVEIRA, R.R.	RENATO RODRIGUES OLIVEIRA	3152
OLIVEIRA, R.T.R.	RENATA TORRES REGO OLIVEIRA	3247
OLIVEIRA, R.T.R.	RENATA TORRES REGO OLIVEIRA	3273
OLIVEIRA, R.T.R.	RENATA TORRES REGO OLIVEIRA	3456
OLIVEIRA, S.C.	SERGIO COSTA OLIVEIRA	2651
OLIVEIRA, S.C.	SERGIO COSTA OLIVEIRA	2781
OLIVEIRA, S.C.	SERGIO COSTA OLIVEIRA	2828
OLIVEIRA, S.C.	SERGIO COSTA OLIVEIRA	3370
OLIVEIRA, S.C.D.A.	STEFANIE CRISTINA DE AZEVEDO OLIVEIRA	3015
OLIVEIRA, S.C.D.A.	STEFANIE CRISTINA DE AZEVEDO OLIVEIRA	3017
OLIVEIRA, S.D.S.	SAMUEL DOS SANTOS OLIVEIRA	2917
OLIVEIRA, S.N.	SAMANTHA NEVES OLIVEIRA	2657
OLIVEIRA, S.N.	SAMANTHA NEVES OLIVEIRA	2675
OLIVEIRA, S.N.	SAMANTHA NEVES OLIVEIRA	2685
OLIVEIRA, S.N.	SAMANTHA NEVES OLIVEIRA	3316
OLIVEIRA, S.N.	SAMANTHA NEVES OLIVEIRA	3434
OLIVEIRA, S.N.	SAMANTHA NEVES OLIVEIRA	3451

OLIVEIRA, T.G.M.	THÉO GREMEN MIMARY OLIVEIRA	2762
OLIVEIRA, T.G.M.	THÉO GREMEN MIMARY OLIVEIRA	2855
OLIVEIRA, V.D.A.	VINÍCIUS DE ARAÚJO OLIVEIRA	3291
OLIVEIRA, Y.D.S.	YASMIN DA SILVA OLIVEIRA	2946
OLIVELLA, J.G.B.	JULIANNA GIORDANO BOTELHO OLIVELLA	2874
OLIVELLA, J.G.B.	JULIANNA GIORDANO BOTELHO OLIVELLA	2911
OLÓRTEGUI, C.D.C.	CARLOS DELFIN CHÁVEZ OLÓRTEGUI	2676
OLSEN, P.	PRISCILLA OLSEN	3090
OLSEN, P.C.	PRISCILLA CHRISTINA OLSEN	2814
OLSEN, P.C.	PRISCILLA CHRISTINA OLSEN	2968
OLSEN, P.C.	PRISCILLA CHRISTINA OLSEN	3095
OLSEN, P.C.	PRISCILLA CHRISTINA OLSEN	3478
OMER, S.	SAAD OMER	2719
O'NEILL, L.	LUKE O'NEILL	3487
ONO, L.A.R.	LAUREN APARECIDA RODRIGUES ONO	2947
ORNELLAS, F.	FELIPE ORNELLAS	2854
ORNELLAS-GARCIA, U.	UYLA ORNELLAS-GARCIA	2786
ORNELLAS-GARCIA, U.	UYLA ORNELLAS-GARCIA	2849
ORRICO-FERREIRA, C.M.	CAROLINA MELO ORRICO-FERREIRA	3182
ORRICO-FERREIRA, C.M.	CAROLINA MELO ORRICO-FERREIRA	3197
ORRICO-FERREIRA, C.M.	CAROLINA MELO ORRICO-FERREIRA	3366
ORSI, R.D.O.	RICARDO DE OLIVEIRA ORSI	3319
ORTEGA, M.M.	MARINA MAZZILLI ORTEGA	2769
ORTEGA, M.M.	MARINA MAZZILLI ORTEGA	2776
ORTS, D.J.B.	DIEGO JOSÉ BELATO ORTS	3450
OSHIRO, F.P.	FLÁVIO PIGNATARO OSHIRO	3317
OTA, V.A.	VICTOR AKIRA OTA	2818
OTA, V.A.	VICTOR AKIRA OTA	2963
OTÁLORA, N.A.	NATHALIA ALARCÓN OTÁLORA	2984
OTÁLORA, N.A.	NATHALIA ALARCÓN OTÁLORA	3073
OTONI, C.D.M.	CAIO DE MOURA OTONI	3165
OTTA, D.A.	DAYANE ANDRIOTTI OTTA	2638
OTTONE, V.D.O.	VINICIUS DE OLIVEIRA OTTONE	2987
OVIDER, I.C.	IAN COSTA OVIDER	2854
OVIDER, I.C.	IAN COSTA OVIDER	2855
OVIDER, I.C.	IAN COSTA OVIDER	3221
OYAMADA, H.A.D.A.	HUGO AKIRITO DE ALMEIDA OYAMADA	3062
OYAMADA, H.A.D.A.	HUGO AKIRITO DE ALMEIDA OYAMADA	3161
OZELO, M.C.	MARGARETH CASTRO OZELO	3360
PACHECO, L.G.C.	LUÍS GUSTAVO CARVALHO PACHECO	2850
PACHECO, T.C.F.	THÁILLA CRISTINA FARIA PACHECO	2766
PACHECO, T.C.F.	THÁILLA CRISTINA FARIA PACHECO	3160
PACHECO, T.C.F.	THÁILLA CRISTINA FARIA PACHECO	3168
PACHECO, T.C.F.	THÁILLA CRISTINA FARIA PACHECO	3180
PACHECO, T.C.F.	THÁILLA CRISTINA FARIA PACHECO	3206
PACINI, C.P.	CAROLINA PAULINO PACINI	2888
PACOLA, D.P.	DENISE PELUSO PACOLA	3476
PADILHA, J.L.	JOSE LUIZ PADILHA	2972
PADOVANI, B.N.	BARBARA NUNES PADOVANI	2917
PAES, I.F.	IZABELA FELICE PAES	2845
PAES, I.F.	IZABELA FELICE PAES	2848
PAITER, K.H.K.	KATHLEEN HELENA KOLODZIEJSKI PAITER	3339
PAIVA, I.M.	ISADORA MARQUES PAIVA	2833
PAIVA, I.M.	ISADORA MARQUES PAIVA	3022
PAIVA, R.B.L.	RAMON BUSON LIMA PAIVA	3044
PAIXAO, P.H.D.M.	PIERRE HENRIQUE DE MENEZES PAIXAO	2671
PAIXAO, P.H.D.M.	PIERRE HENRIQUE DE MENEZES PAIXAO	2681
PAIXAO, P.H.D.M.	PIERRE HENRIQUE DE MENEZES PAIXAO	2688
PALMEIRO, J.K.	JUSSARA KASUKO PALMEIRO	3449

PALOSCHI, M.V.	MAURO VALENTINO PALOSCHI	2971
PALOSCHI, M.V.	MAURO VALENTINO PALOSCHI	2983
PALOSCHI, M.V.	MAURO VALENTINO PALOSCHI	3514
PAMPLONA, T.C.D.D.L.	THAIANA CRISTINA DIAS DE LIMA PAMPLONA	3522
PAMPOLHA, A.F.O.	ANA FLÁVIA OLIVEIRA PAMPOLHA	3408
PANASCO, M.D.S.	MÔNICA DE SOUZA PANASCO	3245
PANETTI, S.	SILVIA PANETTI	2929
PANSONATO, .L.	BRUNA LINS PANSONATO	3126
PANSONATO, .L.	BRUNA LINS PANSONATO	3388
PANTALEÃO, A.	ALEXANDRE PANTALEÃO	2976
PANUCCI, L.A.G.	LUIS ANTONIO GILBERTI PANUCCI	2736
PAOLINI, P.C.	POLIANA CARINA PAOLINI	3285
PARDIM, M.F.	MATHEUS FELIPE PARDIM	2729
PARDIM, M.F.	MATHEUS FELIPE PARDIM	3104
PARDIM, M.F.	MATHEUS FELIPE PARDIM	3180
PARDIM, M.F.	MATHEUS FELIPE PARDIM	3438
PAREDES, B.D.	BRUNO D PAREDES	3329
PARISIO, J.M.	JULIA MARIA PARISIO	3245
PARRA, R.S.	ROGÉRIO SERAFIM PARRA	3160
PARRA, R.S.	ROGÉRIO SERAFIM PARRA	3168
PARRA, R.S.	ROGÉRIO SERAFIM PARRA	3206
PARRA, R.S.	ROGÉRIO SERAFIM PARRA	3381
PARREIRAS, L.G.	LARA GABRIELLA PARREIRAS	2981
PARREIRAS, L.G.	LARA GABRIELLA PARREIRAS	2993
PASCHOAL, D.	DANILO PASCHOAL	3013
PASCHOAL, D.	DANILO PASCHOAL	3299
PASCHOAL, D.	DANILO PASCHOAL	3301
PASCHOALIM, M.E.D.O.	MARIA EDUARDA DE OLIVEIRA PASCHOALIM	3082
PASCHOALIM, M.E.D.O.	MARIA EDUARDA DE OLIVEIRA PASCHOALIM	3102
PASCHOALIM, M.E.D.O.	MARIA EDUARDA DE OLIVEIRA PASCHOALIM	3162
PASCHOALIM, M.E.D.O.	MARIA EDUARDA DE OLIVEIRA PASCHOALIM	3203
PASCOAL, L.B.	LIVIA BITTENCOURT PASCOAL	3067
PASCOAL-XAVIER, M.A.	MARCELO ANTÔNIO PASCOAL-XAVIER	2635
PASCOAL-XAVIER, M.A.	MARCELO ANTÔNIO PASCOAL-XAVIER	2644
PASCOAL-XAVIER, M.A.	MARCELO ANTÔNIO PASCOAL-XAVIER	2652
PASCOAL-XAVIER, M.A.	MARCELO ANTÔNIO PASCOAL-XAVIER	2667
PASQUALI, C.	CHRISTIAN PASQUALI	3321
PASQUALI, C.	CHRISTIAN PASQUALI	3455
PASSAGLIA, R.D.C.A.T.	RITA DE CÁSSIA ALEIXO TOSTES PASSAGLIA	3269
PASSOS, T.D.O.	THÁIS DE OLIVEIRA PASSOS	3150
PASSOS, T.D.O.	THÁIS DE OLIVEIRA PASSOS	3394
PASSOS, T.D.O.	THÁIS DE OLIVEIRA PASSOS	3457
PAULA, P.D.L.	PRISCILA DE LIMA PAULA	3162
PAULI, J.R.	JOSÉ RODRIGO PAULI	2869
PECONICK, A.P.	ANA PAULA PECONICK	3265
PEDRO, R.B.D.S.	RAQUEL BISPO DE SÃO PEDRO	2833
PEDRO, R.B.D.S.	RAQUEL BISPO DE SÃO PEDRO	2871
PEDRO, R.B.D.S.	RAQUEL BISPO DE SÃO PEDRO	2887
PEDRO, R.B.D.S.	RAQUEL BISPO DE SÃO PEDRO	2910
PEDRO, R.B.D.S.	RAQUEL BISPO DE SÃO PEDRO	3025
PEDRO, R.B.D.S.	RAQUEL BISPO DE SÃO PEDRO	3520
PEDROSA, M.L.M.P.	MARIA LUIZA MUNDIM PORTO PEDROSA	3305
PEDROSA, M.L.M.P.	MARIA LUIZA MUNDIM PORTO PEDROSA	3334
PEIXOTO, A.C.F.	ANA CLARA FERNANDES PEIXOTO	2979
PEIXOTO, B.	BÁRBARA PEIXOTO	2956
PEIXOTO, F.D.C.	FABIO DE CARVALHO PEIXOTO	2921
PEIXOTO, F.D.C.	FABIO DE CARVALHO PEIXOTO	3167
PEIXOTO, F.D.C.	FABIO DE CARVALHO PEIXOTO	3209
PEIXOTO, G.A.D.S.	GLAUBER ARAUJO DA SILVA PEIXOTO	2838

PEIXOTO, J.M.	JUAN MIRANDA PEIXOTO	2665
PEIXOTO, J.M.	JUAN MIRANDA PEIXOTO	2677
PEIXOTO, J.M.	JUAN MIRANDA PEIXOTO	2678
PEIXOTO, J.M.	JUAN MIRANDA PEIXOTO	2682
PEIXOTO, L.L.T.D.	LAURA LOPES TRIGO DUARTE PEIXOTO	3096
PEIXOTO, L.L.T.D.	LAURA LOPES TRIGO DUARTE PEIXOTO	3100
PEIXOTO, M.F.D.	MARCO FABRICIO DIAS PEIXOTO	2987
PEIXOTO, M.F.D.	MARCO FABRICIO DIAS PEIXOTO	3051
PELEGRIN, Á.F.	ÁLVARO FORMOSO PELEGRIN	3013
PELEGRIN, Á.F.	ÁLVARO FORMOSO PELEGRIN	3299
PELEGRIN, Á.F.	ÁLVARO FORMOSO PELEGRIN	3301
PELEGRINO, K.	KARLA PELEGRINO	3060
PELLENZ, M.C.M.	MARIA CRISTINA MALLMANN PELLENZ	3254
PENTEADO, L.D.A.	LETÍCIA DE AQUINO PENTEADO	3283
PENTEADO, L.D.A.	LETÍCIA DE AQUINO PENTEADO	3459
PERAÇOLI, J.C.	JOSÉ CARLOS PERAÇOLI	2985
PERAÇOLI, J.C.	JOSÉ CARLOS PERAÇOLI	3222
PERAÇOLI, M.T.S.	MARIA TEREZINHA SERRÃO PERAÇOLI	2985
PERAÇOLI, M.T.S.	MARIA TEREZINHA SERRÃO PERAÇOLI	3222
PERAZZO, H.	HUGO PERAZZO	3105
PERCE-DA-SILVA, D.D.S.	DAIANA DE SOUZA PERCE-DA-SILVA	2743
PERCEGONI, A.C.R.	ANA CLARA RODRIGUES PERCEGONI	3062
PERCEGONI, N.	NATHÉRCIA PERCEGONI	3248
PERCEGONI, N.	NATHÉRCIA PERCEGONI	3280
PERCEGONI, N.	NATHÉRCIA PERCEGONI	3302
PERCEGONI, N.	NATHÉRCIA PERCEGONI	3340
PEREIRA, A.A.S.	AGNES ANTÔNIA SAMPAIO PEREIRA	2667
PEREIRA, A.C.D.P.A.	ANA CAROLINA DE PÁDUA ALPINO PEREIRA	3110
PEREIRA, A.C.M.	ARLINDO CÉSAR MATIAS PEREIRA	3188
PEREIRA, A.M.R.	ANTONIO MARCOS RODRIGUES PEREIRA	3237
PEREIRA, A.N.A.	ANDRESSA NATALY ALMEIDA PEREIRA	2987
PEREIRA, B.C.D.S.	BÁRBARA CRISTINA DA SILVA PEREIRA	3095
PEREIRA, B.T.N.	BRUNA TOLEDO NUNES PEREIRA	2889
PEREIRA, C.D.S.F.	CAROLINA DE SOUZA FARIA PEREIRA	3050
PEREIRA, C.F.D.A.	CAMILA FARIA DE AMORIM PEREIRA	3085
PEREIRA, C.L.	CECILIA LUIZA PEREIRA	2695
PEREIRA, C.L.	CECILIA LUIZA PEREIRA	2696
PEREIRA, C.L.	CECILIA LUIZA PEREIRA	2697
PEREIRA, C.L.	CECILIA LUIZA PEREIRA	2700
PEREIRA, C.L.	CECILIA LUIZA PEREIRA	2703
PEREIRA, C.L.	CECILIA LUIZA PEREIRA	2704
PEREIRA, C.L.D.	CARLOS LORRAN DIAS PEREIRA	2670
PEREIRA, D.	DHÉLIO PEREIRA	2636
PEREIRA, D.	DHÉLIO PEREIRA	2639
PEREIRA, D.	DHÉLIO PEREIRA	2650
PEREIRA, D.D.S.	DANIELE DE SÁ PEREIRA	3323
PEREIRA, E.C.	ELISA CAVALCANTE PEREIRA	3187
PEREIRA, F.K.F.	FRANCISCA KALINE FERREIRA PEREIRA	3133
PEREIRA, G.	GABRIEL PEREIRA	2903
PEREIRA, G.F.P.D.M.	GABRIEL FRANCISCO POZO DE MATTOS PEREIRA	3289
PEREIRA, G.M.B.	GERALDO MOURA BATISTA PEREIRA	3065
PEREIRA, G.M.B.	GERALDO MOURA BATISTA PEREIRA	3371
PEREIRA, G.M.C.D.A.	GUSTAVO MEIRA CHAVES DE ASSIS PEREIRA	3178
PEREIRA, H.G.	HELOÍSA GIRARDI PEREIRA	3387
PEREIRA, H.G.	HELOÍSA GIRARDI PEREIRA	3391
PEREIRA, J.	JULIANNA PEREIRA	3029
PEREIRA, J.	JULIANNA PEREIRA	3171
PEREIRA, J.	JULIANNA PEREIRA	3309
PEREIRA, J.A.	JÉSSICA ASSIS PEREIRA	3180

PEREIRA, K.A.S.	KEISE ADRIELLE SANTOS PEREIRA	3130
PEREIRA, K.A.S.	KEISE ADRIELLE SANTOS PEREIRA	3185
PEREIRA, L.C.V.	LARISSA CARVALHINHA VARANDA PEREIRA	3036
PEREIRA, L.G.G.	LETÍCIA GRILLO GUIMARÃES PEREIRA	3067
PEREIRA, L.G.L.	LUIZA GABRIELE LIMA PEREIRA	3081
PEREIRA, L.G.L.	LUIZA GABRIELE LIMA PEREIRA	3082
PEREIRA, L.G.L.	LUIZA GABRIELE LIMA PEREIRA	3102
PEREIRA, L.G.L.	LUIZA GABRIELE LIMA PEREIRA	3162
PEREIRA, L.M.	LUIZ MIGUEL PEREIRA	3344
PEREIRA, L.M.M.	LUIZA MELO MADUREIRA PEREIRA	3055
PEREIRA, L.M.M.	LUIZA MELO MADUREIRA PEREIRA	3308
PEREIRA, L.M.M.	LUIZA MELO MADUREIRA PEREIRA	3361
PEREIRA, L.P.M.C.	LUDMILA PONCE MONKEN CUSTÓDIO PEREIRA	2834
PEREIRA, M.H.G.	MARCELA HELENA GONÇALVES PEREIRA	2640
PEREIRA, M.M.D.P.	MYLENA MASSENO DE PINHO PEREIRA	3481
PEREIRA, M.P.	MELISSA PONTES PEREIRA	3466
PEREIRA, M.R.	MARIANA RISARDI PEREIRA	2902
PEREIRA, N.V.	NAIURA VIEIRA PEREIRA	3137
PEREIRA, P.H.F.	PEDRO HENRIQUE FERNANDES PEREIRA	3246
PEREIRA, R.A.D.S.	ROSIANE APARECIDA DA SILVA PEREIRA	2638
PEREIRA, R.A.D.S.	ROSIANE APARECIDA DA SILVA PEREIRA	2646
PEREIRA, R.A.D.S.	ROSIANE APARECIDA DA SILVA PEREIRA	2660
PEREIRA, R.C.D.S.	RAYANE CAROLINE DOS SANTOS PEREIRA	3522
PEREIRA, R.D.D.	RAFAELA DAS DORES PEREIRA	2701
PEREIRA, R.D.D.	RAFAELA DAS DORES PEREIRA	2702
PEREIRA, R.D.M.S.	RENATA DE MEIRELLES SANTOS PEREIRA	2956
PEREIRA, R.D.M.S.	RENATA DE MEIRELLES SANTOS PEREIRA	3150
PEREIRA, R.D.M.S.	RENATA DE MEIRELLES SANTOS PEREIRA	3394
PEREIRA, R.D.M.S.	RENATA DE MEIRELLES SANTOS PEREIRA	3457
PEREIRA, S.D.S.	SORAYA DOS SANTOS PEREIRA	2971
PEREIRA, S.D.S.	SORAYA DOS SANTOS PEREIRA	3514
PEREIRA, T.B.S.	TAMIRES BOMFIM SANTOS PEREIRA	3154
PEREIRA, V.A.C.	VINÍCIUS ALEX CANO PEREIRA	2855
PEREIRA, V.A.C.	VINÍCIUS ALEX CANO PEREIRA	3226
PEREIRA, V.A.C.	VINÍCIUS ALEX CANO PEREIRA	3236
PEREIRA, V.S.	VERÔNICA SCHMITZ PEREIRA	3228
PEREIRA, V.S.D.A.	VERONICA SCHINAIDER DO AMARAL PEREIRA	3100
PEREIRA, W.G.F.	WILIAN GOMES FERNANDES PEREIRA	2839
PEREIRA-DUTRA, F.	FILIPE PEREIRA-DUTRA	3043
PEREIRA-DUTRA, F.	FILIPE PEREIRA-DUTRA	3179
PEREIRA-DUTRA, F.	FILIPE PEREIRA-DUTRA	3279
PEREIRA-DUTRA, F.	FILIPE PEREIRA-DUTRA	3439
PERES, A.	ALESSANDRA PERES	2826
PERES, R.S.	RAPHAEL S. PERES	3160
PERES, R.S.	RAPHAEL S. PERES	3168
PERES, R.S.	RAPHAEL S. PERES	3206
PEREZ, G.G.	GUSTAVO GUADAGNINI PEREZ	3346
PEREZ, Y.G.	YENLY GONZALEZ PEREZ	2727
PERNAMBUCO FILHO, P.C.D.A.	PAULO CASTANHO DE ALMEIDA PERNAMBUCO FILHO	2835
PERON, J.P.S.	JEAN PIERRE SCHATZMANN PERON	2958
PERRETTI, M.	MAURO PERRETTI	3329
PERUHYPE-MAGALHÃES, V.	VANESSA PERUHYPE-MAGALHÃES	2635
PERUHYPE-MAGALHÃES, V.	VANESSA PERUHYPE-MAGALHÃES	2638
PERUHYPE-MAGALHÃES, V.	VANESSA PERUHYPE-MAGALHÃES	2649
PERUSSOLO, M.C.	MAIARA CAROLINA PERUSSOLO	2857
PESSOA, C.A.	CAROLINY ALVES PESSOA	2683
PESSOLANI, M.C.V.	MARIA CRISTINA VIDAL PESSOLANI	3228
PESSOLANI, M.C.V.	MARIA CRISTINA VIDAL PESSOLANI	3237
PESSOLANI, M.C.V.	MARIA CRISTINA VIDAL PESSOLANI	3466

PESTANA, J.D.C.	JÚLIA DA CONCEIÇÃO PESTANA	2941
PESTANA, J.D.C.	JÚLIA DA CONCEIÇÃO PESTANA	2977
PETRILLI, J.D.	JÉSSICA DIAS PETRILLI	2946
PFEFER, T.	THOMAS PFEFER	3487
PHELIPPE, N.E.	NICOLE ERCULANA PHELIPPE	2879
PIAU, B.C.	BRUNA COSTA PIAU	2641
PIAUY, M.H.S.	MEIRE HELLEN SANTOS PIAUY	3466
PIAZI, M.D.	MARCELLE DRUMOND PIAZI	3184
PIAZI, M.D.	MARCELLE DRUMOND PIAZI	3187
PIAZI, M.D.	MARCELLE DRUMOND PIAZI	3249
PICCIN, M.P.C.	MARIELA PIRES CABRAL PICCIN	2841
PICCOO, R.L.	RAFAEL LEITE PICCOO	3201
PIERRE, M.L.M.	MARIA LUISA MARQUES PIERRE	2827
PIERRE, M.L.M.	MARIA LUISA MARQUES PIERRE	2841
PIERRE, M.L.M.	MARIA LUISA MARQUES PIERRE	3094
PIERRE, M.L.M.	MARIA LUISA MARQUES PIERRE	3171
PIERRE, M.L.M.	MARIA LUISA MARQUES PIERRE	3309
PIETROBON, A.J.	ANNA JULIA PIETROBON	2730
PIGATTO, G.R.	GLAUCE REGINA PIGATTO	3054
PILGER, L.M.	LETICIA MARQUES PILGER	3250
PILGER, L.M.	LETICIA MARQUES PILGER	3298
PILGER, L.M.	LETICIA MARQUES PILGER	3464
PILLI, R.A.	RONALDO ALOISE PILLI	3252
PIMENTA, A.J.	ANNA JULYA PIMENTA	2897
PIMENTA, A.J.	ANNA JULYA PIMENTA	3099
PIMENTA, A.J.	ANNA JULYA PIMENTA	3132
PIMENTA, A.J.	ANNA JULYA PIMENTA	3138
PIMENTA, A.J.	ANNA JULYA PIMENTA	3195
PIMENTA, A.J.	ANNA JULYA PIMENTA	3238
PIMENTA, A.J.	ANNA JULYA PIMENTA	3241
PIMENTA, A.J.	ANNA JULYA PIMENTA	3256
PIMENTA, A.L.B.	ANA LUÍSA BATISTA PIMENTA	2987
PIMENTA, A.L.B.	ANA LUÍSA BATISTA PIMENTA	3165
PIMENTA, B.C.	BRUNA CAETANO PIMENTA	3227
PIMENTA, B.C.	BRUNA CAETANO PIMENTA	3409
PIMENTA, D.C.	DANIEL CARVALHO PIMENTA	3326
PIMENTA, J.V.	JULIANA VIEIRA PIMENTA	2795
PIMENTA, J.V.	JULIANA VIEIRA PIMENTA	2796
PIMENTEL, I.H.M.	ITALO HUGO MACIEL PIMENTEL	2900
PINHATE, S.B.	SYLVIA BARBOSA PINHATE	3053
PINHATE, S.B.	SYLVIA BARBOSA PINHATE	3389
PINHEIRO, C.D.S.	CARINA DA SILVA PINHEIRO	2850
PINHEIRO, C.D.S.	CARINA DA SILVA PINHEIRO	3182
PINHEIRO, C.D.S.	CARINA DA SILVA PINHEIRO	3197
PINHEIRO, C.D.S.	CARINA DA SILVA PINHEIRO	3366
PINHEIRO, C.O.D.D.	CAIO OMORI DEL DEBBIO PINHEIRO	2879
PINHEIRO, C.O.D.D.	CAIO OMORI DEL DEBBIO PINHEIRO	2902
PINHEIRO, D.F.	DOUGLAS FLORINDO PINHEIRO	3245
PINHEIRO, F.M.	FERNANDA MARTINS PINHEIRO	2834
PINHEIRO, L.S.	LILIANE SENA PINHEIRO	3051
PINHEIRO, M.	MARCOS PINHEIRO	3219
PINHEIRO, M.C.M.	MARIA CLARA MOURA PINHEIRO	3103
PINHEIRO, M.C.M.	MARIA CLARA MOURA PINHEIRO	3190
PINHEIRO, R.D.S.	REBECA DE SOUZA PINHEIRO	3083
PINHEIRO, R.D.S.	REBECA DE SOUZA PINHEIRO	3272
PINHEIRO, R.O.	ROBERTA OLMO PINHEIRO	3087
PINHEIRO, R.O.	ROBERTA OLMO PINHEIRO	3237
PINHEIRO, R.O.	ROBERTA OLMO PINHEIRO	3481
PINHO, N.	NATHALIA PINHO	3009

PINTO, A.R.	AGUINALDO R PINTO	3387
PINTO, A.R.	AGUINALDO R PINTO	3391
PINTO, A.R.	ANA RAQUEL PINTO	2719
PINTO, C.H.R.	CECILIA HORTA RAMALHO PINTO	2656
PINTO, C.H.R.	CECILIA HORTA RAMALHO PINTO	2676
PINTO, C.H.R.	CECILIA HORTA RAMALHO PINTO	2684
PINTO, E.F.	EDUARDO F PINTO	2823
PINTO, K.G.	KAMILA GUIMARÃES PINTO	2897
PINTO, K.G.	KAMILA GUIMARÃES PINTO	3099
PINTO, K.G.	KAMILA GUIMARÃES PINTO	3132
PINTO, K.G.	KAMILA GUIMARÃES PINTO	3138
PINTO, K.G.	KAMILA GUIMARÃES PINTO	3195
PINTO, K.G.	KAMILA GUIMARÃES PINTO	3232
PINTO, K.G.	KAMILA GUIMARÃES PINTO	3238
PINTO, K.G.	KAMILA GUIMARÃES PINTO	3241
PINTO, K.G.	KAMILA GUIMARÃES PINTO	3256
PINTO, L.	LETÍCIA PINTO	2974
PINTO, L.F.	LARISSA FAGUNDES PINTO	2759
PINTO, N.D.S.	NOME DE SOUZA PINTO	3183
PINTO, N.D.S.	NOME DE SOUZA PINTO	3250
PINTO, N.V.	NATALHA VICENTINA PINTO	3414
PINTOR, F.A.	FERNANDA ARAÚJO PINTOR	3309
PINZAN, C.F.	CAMILA FIGUEIREDO PINZAN	3080
PIRAINE3, R.E.A.	RENAN EUGÊNIO ARAUJO PIRAINÉ3	2808
PIRES, A.D.S.	ARMANDO DA SILVA PIRES	3211
PIRES, A.D.S.	ARMANDO DA SILVA PIRES	3279
PIRES, G.M.M.	GEOVANNA MARIA MALACHIAS PIRES	3146
PIRES, G.M.M.	GEOVANNA MARIA MALACHIAS PIRES	3202
PIRES, M.M.D.S.	MARIA MARLENE DE SOUZA PIRES	3449
PIRES, T.R.C.	THYAGO RUBENS CARDIM PIRES	3278
PISCHEL, L.	LAUREN PISCHEL	2719
PISCULICH, D.C.B.	DANIELLA CHRISTINA BERTOLOTTI PISCULICH	3228
PISCULICH, D.C.B.	DANIELLA CHRISTINA BERTOLOTTI PISCULICH	3466
PITOLI, B.D.S.	BEATRIZ DE SOUZA PITOLI	2869
PITTELLA, F.	FABIO PITTELLA	3510
PIVETTA, R.S.	RENATA SCHMIEDER PIVETTA	2821
PIVETTA, R.S.	RENATA SCHMIEDER PIVETTA	2896
PIVETTA, R.S.	RENATA SCHMIEDER PIVETTA	3039
POEYS, A.C.D.S.	ANA CLARA DA SILVA POEYS	2786
POEYS, A.C.D.S.	ANA CLARA DA SILVA POEYS	2849
POLIDO, V.P.	VICTOR P. POLIDO	2927
POLIDORO, J.Z.	JULIANO ZEQUINI POLIDORO	2975
POLITO, E.F.R.	EDUARDA FERREIRA ROCHA POLITO	2759
POMBO, F.C.D.N.	FRANCISCO CÂNDIDO DO NASCIMENTO POMBO	2945
POMBO-DE-OLIVEIRA, M.S.	MARIA S. POMBO-DE-OLIVEIRA	3151
POMBO-DE-OLIVEIRA, M.S.	MARIA S. POMBO-DE-OLIVEIRA	3268
PONCE, M.	MARCELA PONCE	3135
PONTE, M.S.G.	MELISSA SILVA GONÇALVES PONTE	3356
PONTES, G.S.	GEMILSON SOARES PONTES	3445
PONTES, J.K.	JULIO KORNETZ PONTES	3090
PONTES, L.L.D.F.	LORENA LOBO DE FIGUEIREDO PONTES	3285
PONTILLO, A.	ALESSANDRA PONTILLO	2767
PONTILLO, A.	ALESSANDRA PONTILLO	3091
PONTILLO-GUIMARÃES, G.	GIOVANA PONTILLO-GUIMARÃES	3065
POPI, A.F.	ANA FLAVIA POPI	2789
POPI, A.F.	ANA FLAVIA POPI	3419
POPPE, J.G.	JULIANA GORGEN POPPE	3321
POPPE, J.G.	JULIANA GORGEN POPPE	3455
PORCINO, G.N.	GABRIANE NASCIMENTO PORCINO	2980

PORCINO, G.N.	GABRIANE NASCIMENTO PORCINO	3363
PORTELA, J.V.R.	JÚLIA VITÓRIA ROMA PORTELA	3062
PORTELA, L.F.	LUCIANA FERNANDES PORTELA	3359
PORTELA, L.F.	LUCIANA FERNANDES PORTELA	3404
PORTES JUNIOR, J.A.	JOSÉ ANTÔNIO PORTES JUNIOR	3218
PORTES JUNIOR, J.A.	JOSÉ ANTÔNIO PORTES JUNIOR	3277
PORTILHO, A.I.	AMANDA I PORTILHO	3450
PORTILHO, A.I.	AMANDA IZELI PORTILHO	3002
PORTILHO, A.I.	AMANDA IZELI PORTILHO	3042
PORTILHO, A.I.	AMANDA IZELI PORTILHO	3061
PORTO, B.N.	BARBARA NERY PORTO	3435
PORTO, H.F.B.	HELENA FERREIRA BRUZZI PORTO	2927
POSSIK, P.A.	PATRICIA ABRÃO POSSIK	3446
POSTOL, E.	EDILBERTO POSTOL	3149
POTENZA, A.D.S.	ANDRE DE SOUZA POTENZA	3133
PÓVOAS, D.	DIANA PÓVOAS	2719
PRADO, C.M.	CARLA MÁXIMO PRADO	3076
PRADO, H.	HELOISA PRADO	3004
PRADO, J.D.A.	JULIANA DE ALMEIDA PRADO	3297
PRADO, N.D.R.	NIDIANE DANTAS REIS PRADO	3514
PRADO, R.O.	ROBERTA OLIVEIRA PRADO	2667
PRAL, L.P.	LAÍS PASSARIELO PRAL	2798
PRATA, M.A.	MARCIA AZEVEDO PRATA	3100
PRAXEDES, I.R.	ISABELE RODRIGUES PRAXEDES	3083
PRAXEDES, I.R.	ISABELE RODRIGUES PRAXEDES	3272
PRAZERES, P.H.D.M.	PEDRO HENRIQUE DIAS MOURA PRAZERES	2673
PREITE, N.W.	NYCOLAS WILLIAN PREITE	3515
PRESTES, E.B.	ELISA BEATRIZ PRESTES	3253
PRESTON, H.	HANNAH PRESTON	2978
PREVIATO, J.O.	JOSÉ OSVALDO PREVIATO	2738
PREVIATO, J.O.	JOSÉ OSVALDO PREVIATO	2912
PREVIATO, J.O.	JOSÉ OSVALDO PREVIATO	2948
PREVIATO, J.O.	JOSÉ OSVALDO PREVIATO	3418
PREVIATO, L.M.	LUCIA MENDONÇA PREVIATO	2738
PREVIATO, L.M.	LUCIA MENDONÇA PREVIATO	2912
PREVIATO, L.M.	LUCIA MENDONÇA PREVIATO	2948
PREVIATO, L.M.	LUCIA MENDONÇA PREVIATO	3418
PROOST, P.	PAUL PROOST	2846
PRUDENCIO, C.R.	CARLOS ROBERTO PRUDENCIO	2906
PRUDENCIO, C.R.	CARLOS ROBERTO PRUDENCIO	3002
PRUDENCIO, C.R.	CARLOS ROBERTO PRUDENCIO	3042
PRUDENCIO, C.R.	CARLOS ROBERTO PRUDENCIO	3096
PRUDENCIO, C.R.	CARLOS ROBERTO PRUDENCIO	3450
PSCHEIDT, V.M.	VERIDIANE MARIA PSCHIEDT	3031
PSCHEIDT, V.M.	VERIDIANE MARIA PSCHIEDT	3353
PÚBLIO, G.A.	GABRIEL AZEVEDO PÚBLIO	3333
PÚBLIO, G.A.	GABRIEL AZEVEDO PÚBLIO	3415
PUCCA, M.B.	MANUELA BERTO PUCCA	3146
PUCCA, M.B.	MANUELA BERTO PUCCA	3163
PUCCA, M.B.	MANUELA BERTO PUCCA	3173
PUCCA, M.B.	MANUELA BERTO PUCCA	3201
PUCCA, M.B.	MANUELA BERTO PUCCA	3202
PUERNHAGEN, L.R.P.	LOUISE RUBIA PROBST PUERNHAGEN	3300
PYRRHO, A.D.S.	ALEXANDRE DOS SANTOS PYRRHO	3070
PYRRHO, A.D.S.	ALEXANDRE DOS SANTOS PYRRHO	3109
QUADROS-PEREIRA, L.	LAURA QUADROS-PEREIRA	2909
QUADROS-PEREIRA, L.	LAURA QUADROS-PEREIRA	2917
QUAGLIATO, E.N.	EMMANUELLE NASCIMENTO QUAGLIATO	3077
QUEIROZ JUNIOR, C.	CELSO QUEIROZ JUNIOR	2657

QUEIROZ JUNIOR, C.	CELSO QUEIROZ JUNIOR	2675
QUEIROZ JUNIOR, C.	CELSO QUEIROZ JUNIOR	2686
QUEIROZ JUNIOR, C.	CELSO QUEIROZ JUNIOR	2701
QUEIROZ JUNIOR, C.	CELSO QUEIROZ JUNIOR	2988
QUEIROZ JUNIOR, C.	CELSO QUEIROZ JUNIOR	3316
QUEIROZ JUNIOR, C.	CELSO QUEIROZ JUNIOR	3434
QUEIROZ, A.M.V.	ALINE MARIA VASCONCELOS QUEIROZ	3453
QUEIROZ, F.R.	FÁBIO RIBEIRO QUEIROZ	2635
QUEIROZ, M.G.A.	MILENE GABRIELE ALVES QUEIROZ	2782
QUEIROZ, R.F.G.E.	RAFAELLA FORTINI GRENFELL E QUEIROZ	2756
QUEIROZ, R.F.G.E.	RAFAELLA FORTINI GRENFELL E QUEIROZ	3267
QUEIROZ, R.F.G.E.	RAFAELLA FORTINI GRENFELL E QUEIROZ	3294
QUEIROZ, W.D.M.	WEMERSON DE MORAIS QUEIROZ	2987
QUEIROZ, W.D.M.	WEMERSON DE MORAIS QUEIROZ	3165
QUESNE, A.H.M.L.	ANDRÉS HERNAN MOJOLI LE QUESNE	2872
QUESNE, A.H.M.L.	ANDRÉS HERNAN MOJOLI LE QUESNE	3009
QUIARIM, T.M.	TALITA MOTTA QUIARIM	2819
QUINTANA, M.S.	MARCEL SOUZA QUINTANA	3404
QUINTERO PAMPLONA, G.	GIOVANNA QUINTERO PAMPLONA	3522
QUINTILIO, W.	WAGNER QUINTILIO	3351
QUIRINO, W.G.	WELBER GIANINI QUIRINO	3336
RABELLO, M.I.D.S.	MARIA ISABEL DE SAMPAIO RABELLO	2842
RABELLO, T.	TAILENE RABELLO	3036
RABELO, M.X.	MARJORY XIMENES RABELO	3445
RABELO, R.A.N.	RAYANE APARECIDA NONATO RABELO	2699
RABELO, R.A.N.	RAYANE APARECIDA NONATO RABELO	2701
RAEDER, P.H.L.	PAULO HENRIQUE LISBOA RAEDER	2989
RAGHAVAN, V.	VARSHA RAGHAVAN	3086
RAGONESE, F.	FERNANDA RAGONESE	3283
RAHMAN, A.U.	ATTA UR RAHMAN	3481
RAIA, S.M.	SILVANO MA RAIA	3093
RAIA, S.M.	SILVANO MA RAIA	3101
RAIMUNDO, B.V.B.	BRENO VILAS BOAS RAIMUNDO	3189
RAIMUNDO, B.V.B.	BRENO VILAS BOAS RAIMUNDO	3258
RAIMUNDO, B.V.B.	BRENO VILAS BOAS RAIMUNDO	3283
RAIMUNDO, B.V.B.	BRENO VILAS BOAS RAIMUNDO	3459
RAIMUNDO, B.V.B.	BRENO VILAS BOAS RAIMUNDO	3460
RAINER, M.N.D.	MIGUEL NOLASCO DANTAS RAINER	2663
RAJÃO, M.	MATHEUS RAJÃO	3329
RALPH, A.C.L.	ANA CAROLINA LIMA RALPH	3040
RAMBO, D.F.	DOUGLAS FERNANDO RAMBO	3406
RAMIREZ, J.A.Z.	JOFER ANDREE ZAMAME RAMIREZ	2645
RAMIREZ, J.A.Z.	JOFER ANDREE ZAMAME RAMIREZ	2664
RAMÍREZ, J.D.	JUAN DAVID RAMÍREZ	2630
RAMOS, A.	ALICE RAMOS	2816
RAMOS, A.	ALICE RAMOS	2998
RAMOS, A.	ALICE RAMOS	3006
RAMOS, A.D.S.	ANDERSON DOS SANTOS RAMOS	3160
RAMOS, A.D.S.	ANDERSON DOS SANTOS RAMOS	3168
RAMOS, A.D.S.	ANDERSON DOS SANTOS RAMOS	3206
RAMOS, A.D.S.	ANDERSON DOS SANTOS RAMOS	3269
RAMOS, A.D.S.	ANDERSON DOS SANTOS RAMOS	3384
RAMOS, A.D.S.	ANDERSON DOS SANTOS RAMOS	3408
RAMOS, B.V.S.P.	BÁRBARA VAN STEEN PRONER RAMOS	2922
RAMOS, C.D.L.	CAIO DE LUCENA RAMOS	3106
RAMOS, C.D.L.	CAIO DE LUCENA RAMOS	3239
RAMOS, C.H.I.	CARLOS HENRIQUE INÁCIO RAMOS	2732
RAMOS, D.	DAYANE RAMOS	3482
RAMOS, E.D.S.	EMILLY DOS SANTOS RAMOS	3383

RAMOS, G.P.	GABRIEL PEREIRA RAMOS	3305
RAMOS, G.P.	GABRIEL PEREIRA RAMOS	3334
RAMOS, K.D.S.	KAMILA DE SOUZA RAMOS	3183
RAMOS, L.G.	LAÍS GOMES RAMOS	2686
RAMOS, L.M.G.	LILIAN MARIA GARCIA RAMOS	3285
RAMOS, P.E.R.	PAULA ELISA RODRIGUES RAMOS	3046
RAMOS, P.I.	PABLO IVAN RAMOS	3508
RAMOS, R.B.	RAFAEL BOCAMINO RAMOS	2962
RAMOS, R.C.F.	REGINA C. F. RAMOS	2833
RAMOS, R.N.	RODRIGO NALIO RAMOS	2762
RAMOS, R.N.	RODRIGO NALIO RAMOS	2854
RAMOS, R.N.	RODRIGO NALIO RAMOS	2855
RAMOS, R.N.	RODRIGO NALIO RAMOS	3040
RAMOS, R.N.	RODRIGO NALIO RAMOS	3077
RAMOS, R.N.	RODRIGO NALIO RAMOS	3221
RAMOS, R.N.	RODRIGO NALIO RAMOS	3226
RAMOS, R.N.	RODRIGO NALIO RAMOS	3236
RAMOS, T.D.	TADEU DINIZ RAMOS	3507
RAMOS, Y.Á.L.	YASMIM ÁLEFE LEUZZI RAMOS	3137
RAMPINELLI, P.G.	POLLIANNE GARBERO RAMPINELLI	3352
RANGEL, B.A.B.	BÁRBARA ANDRADE BARCELOS RANGEL	2986
RANGEL-FERREIRA, M.V.	MARCOS VINICIUS RANGEL-FERREIRA	2786
RANGON, S.	SARAH RANGON	2645
RAPOSO, L.M.	LETÍCIA MARTINS RAPOSO	2941
RAPOSO, L.M.	LETÍCIA MARTINS RAPOSO	2977
RAQUEL, L.L.S.	LAÍSSA LOPES SANTANA RAQUEL	3405
RATTIS, B.	BRUNA RATTIS	3428
REAL, F.R.O.	FERNANDO ROBERTO OLIVEIRA REAL	3417
REBELATO, B.	BRUNA REBELATO	3251
REGEN, T.	TOMMY REGEN	3269
REINHARDT, G.M.	GIULIA MENCARINI REINHARDT	3218
REIS, A.C.C.	ANA CAROLINA CRUZ REIS	3102
REIS, A.C.C.	ANA CAROLINA CRUZ REIS	3147
REIS, A.P.G.	ANA PAULA GOULART REIS	2987
REIS, A.P.G.	ANA PAULA GOULART REIS	3165
REIS, B.S.	BERNARDO SGARBI REIS	2798
REIS, D.S.	DANIELA S REIS	2640
REIS, E.V.D.S.	ERIK VINICIUS DE SOUSA REIS	2714
REIS, E.V.D.S.	ERIK VINICIUS DE SOUSA REIS	3251
REIS, I.B.	IANNY BRUM REIS	3013
REIS, I.B.	IANNY BRUM REIS	3299
REIS, I.B.	IANNY BRUM REIS	3301
REIS, L.	LUCIANA REIS	3187
REIS, L.D.C.	LUIZA DE CAMPOS REIS	3281
REIS, L.R.	LAISE RODRIGUES REIS	2649
REIS, M.O.	MARCELLA OLIVEIRA REIS	3081
REIS, M.O.	MARCELLA OLIVEIRA REIS	3082
REIS, M.O.	MARCELLA OLIVEIRA REIS	3102
REIS, M.O.	MARCELLA OLIVEIRA REIS	3162
REIS, P.A.	PATRICIA ALVES REIS	2837
REIS, P.A.	PATRICIA ALVES REIS	3015
REIS, P.A.	PATRICIA ALVES REIS	3017
REIS, P.A.	PATRICIA ALVES REIS	3055
REIS, P.A.	PATRICIA ALVES REIS	3257
REIS, P.A.	PATRICIA ALVES REIS	3279
REIS, P.A.	PATRICIA ALVES REIS	3308
REIS, P.A.	PATRICIA ALVES REIS	3310
REIS, P.A.	PATRICIA ALVES REIS	3361
REIS-CUNHA, J.L.	JOÃO LUÍS REIS-CUNHA	2651

REITER, K.C.	KELI CRISTINE REITER	3186
RENAULT, L.Z.	LAURA ZALCBERG RENAULT	2963
RENOVATO-MARTINS, M.	MARIANA RENOVATO-MARTINS	2925
RENZETTI, A.R.D.S.	ALINNE RANGEL DOS SANTOS RENZETTI	3359
RENZETTI, A.R.D.S.	ALINNE RANGEL DOS SANTOS RENZETTI	3404
REQUIAO, R.D.	RODRIGO DIAS REQUIAO	2809
REQUIAO, R.D.	RODRIGO DIAS REQUIAO	2831
REQUIAO, R.D.	RODRIGO DIAS REQUIAO	3004
REQUIAO, R.D.	RODRIGO DIAS REQUIAO	3487
RESENDE, A.L.D.S.	ANA LUIZA DA SILVA RESENDE	2790
RESENDE, L.M.	LARISSA MEIRA RESENDE	2660
RESENDE, P.C.	PAOLA CRISTINA RESENDE	3187
RESENDE, R.D.O.	RAFAEL DE OLIVEIRA RESENDE	2705
RETES, H.M.	HENRIQUE MORAIS RETES	2670
RETES, H.M.	HENRIQUE MORAIS RETES	2692
REZENDE, I.C.	ISABELLA CONSTANTINO REZENDE	2699
REZENDE, R.R.	RAISSA RAMINELLI REZENDE	2870
RIBEIRO FILHO, O.G.	ORLANDO GARCIA RIBEIRO FILHO	2894
RIBEIRO JUNIOR, R.S.	ROBERTO SILVA RIBEIRO JUNIOR	3403
RIBEIRO, A.	ANTONIO RIBEIRO	2972
RIBEIRO, Á.L.	ÁGATA LOPES RIBEIRO	2670
RIBEIRO, Á.L.	ÁGATA LOPES RIBEIRO	2687
RIBEIRO, Á.L.	ÁGATA LOPES RIBEIRO	2690
RIBEIRO, Á.L.	ÁGATA LOPES RIBEIRO	2691
RIBEIRO, Á.L.	ÁGATA LOPES RIBEIRO	2692
RIBEIRO, Á.L.	ÁGATA LOPES RIBEIRO	2693
RIBEIRO, Á.L.	ÁGATA LOPES RIBEIRO	2714
RIBEIRO, B.M.	BERGMANN MORAIS RIBEIRO	3001
RIBEIRO, B.S.A.	BEATRIZ SARA ANELLI RIBEIRO	3523
RIBEIRO, B.V.	BRUNO VINAGRE RIBEIRO	2775
RIBEIRO, B.V.	BRUNO VINAGRE RIBEIRO	3075
RIBEIRO, B.V.	BRUNO VINAGRE RIBEIRO	3156
RIBEIRO, B.V.	BRUNO VINAGRE RIBEIRO	3318
RIBEIRO, B.V.	BRUNO VINAGRE RIBEIRO	3520
RIBEIRO, C.L.G.	CAMILA LAÍS GONÇALVES RIBEIRO	2857
RIBEIRO, C.T.D.	CLAUDIO TADEU DANIEL RIBEIRO	3050
RIBEIRO, D.G.	DAIANE GONZAGA RIBEIRO	2839
RIBEIRO, E.J.	ESTHER JACCOUD RIBEIRO	3399
RIBEIRO, F.	FABIOLA RIBEIRO	2937
RIBEIRO, F.	FABIOLA RIBEIRO	3474
RIBEIRO, H.C.G.	HANNA CAROLINA GOMES RIBEIRO	2823
RIBEIRO, H.C.G.	HANNA CAROLINA GOMES RIBEIRO	3363
RIBEIRO, I.B.D.O.	ISADORA BATISTA DE OTERO RIBEIRO	3053
RIBEIRO, I.B.D.O.	ISADORA BATISTA DE OTERO RIBEIRO	3152
RIBEIRO, I.S.	ISADORA SANTANA RIBEIRO	2655
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	2897
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	3099
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	3132
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	3138
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	3195
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	3238
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	3241
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	3256
RIBEIRO, J.D.O.	JULIANA DE OLIVEIRA RIBEIRO	3261
RIBEIRO, L.B.	LUAN BARBOSA RIBEIRO	3362
RIBEIRO, L.B.	LUAN BARBOSA RIBEIRO	3476
RIBEIRO, L.R.	LAURA RODRIGUES RIBEIRO	2960
RIBEIRO, L.R.R.	LAURA RAQUEL RIOS RIBEIRO	3262
RIBEIRO, M.B.	MARCOS BORGES RIBEIRO	3282

RIBEIRO, M.L.	MARIO LUIZ RIBEIRO	2998
RIBEIRO, M.P.D.	MARIA PIA DINIZ RIBEIRO	3000
RIBEIRO, M.P.D.	MARIA PIA DINIZ RIBEIRO	3105
RIBEIRO, M.P.D.	MARIA PIA DINIZ RIBEIRO	3240
RIBEIRO, M.P.D.	MARIA PIA DINIZ RIBEIRO	3260
RIBEIRO, N.D.A.R.	NAIARA DE ASSIS RABELO RIBEIRO	2685
RIBEIRO, P.D.A.C.	PAULA DO AMARAL COSTA RIBEIRO	2762
RIBEIRO, P.D.A.C.	PAULA DO AMARAL COSTA RIBEIRO	3040
RIBEIRO, R.D.S.	ROBERTA DOS SANTOS RIBEIRO	2685
RIBEIRO, R.D.S.	ROBERTA DOS SANTOS RIBEIRO	3474
RIBEIRO, V.G.	VICTOR GOMES RIBEIRO	3316
RIBEIRO, V.G.	VICTOR GOMES RIBEIRO	3451
RIBEIRO, W.	WILLIAN RIBEIRO	3478
RIBEIRO, W.R.	WILLIAN RODRIGUES RIBEIRO	3422
RIBEIRO-ALMEIDA, M.L.	MÔNICA L. RIBEIRO-ALMEIDA	2786
RIBEIRO-ALMEIDA, M.L.	MÔNICA L. RIBEIRO-ALMEIDA	2849
RIBOVSKI, L.	LAÍS RIBOVSKI	2895
RICCI, M.F.	MAYRA FERNANDA RICCI	2651
RICCI, M.F.	MAYRA FERNANDA RICCI	2698
RICCI, M.F.	MAYRA FERNANDA RICCI	2699
RICCI, M.F.	MAYRA FERNANDA RICCI	2701
RICCI, M.F.	MAYRA FERNANDA RICCI	2702
RICCI, M.F.	MAYRA FERNANDA RICCI	2768
RICCI, M.F.	MAYRA FERNANDA RICCI	3470
RICCIO, E.K.P.	EVELYN KETY PRATT RICCIO	2743
RICCIO, E.K.P.	EVELYN KETY PRATT RICCIO	2893
RICCIO, L.R.P.	LILIAN ROSE PRATT RICCIO	2893
RICCIO, L.R.P.	LILIAN ROSE PRATT RICCIO	3050
RICOTTA, T.Q.N.	TIAGO QUEIROGA NERY RICOTTA	2655
RICOTTA, T.Q.N.	TIAGO QUEIROGA NERY RICOTTA	2661
RICOTTA, T.Q.N.	TIAGO QUEIROGA NERY RICOTTA	2669
RIGONI, T.D.S.	THAÍS DA SILVA RIGONI	3376
RIGONI, T.D.S.	THAÍS DA SILVA RIGONI	3428
RIMKUTE, I.	INGA RIMKUTE	2715
RINCK JUNIOR, J.A.	JOSE AUGUSTO RINCK JUNIOR	3171
RIOS, J.V.B.	JOÃO VITOR BORGES RIOS	3182
RIOS, W.M.	WENDY MARTIN RIOS	2729
RIOS, W.M.	WENDY MARTIN RIOS	3104
RIOS, W.M.	WENDY MARTIN RIOS	3180
RIOS, W.M.	WENDY MARTIN RIOS	3438
RITEAU, N.	NICOLAS RITEAU	2970
RITEAU, N.	NICOLAS RITEAU	3337
RITEAU, N.	NICOLAS RITEAU	3386
RIVERA, K.	KARLIANIE RIVERA	3027
RIZZO, L.	LUCAS RIZZO	3065
RIZZO, L.V.	LUIZ VICENTE RIZZO	2777
ROBERTO, S.A.	SIBELE ANDRADE ROBERTO	3514
ROBSON, S.C.	SIMON C ROBSON	3008
ROBSON, S.C.	SIMON C ROBSON	3230
ROBSON, S.C.	SIMON C ROBSON	3325
ROCHA, A.C.	ALICE CEDROLA ROCHA	3087
ROCHA, B.	BIANCA ROCHA	2717
ROCHA, F.E.O.	FELIPE EMANUEL OLIVEIRA ROCHA	2686
ROCHA, F.E.O.	FELIPE EMANUEL OLIVEIRA ROCHA	3421
ROCHA, G.D.	GABRIELA D ROCHA	2833
ROCHA, I.	ISABELA ROCHA	2732
ROCHA, I.A.D.C.	ISMAEL ARTUR DA COSTA ROCHA	2638
ROCHA, I.A.D.C.	ISMAEL ARTUR DA COSTA ROCHA	2649
ROCHA, I.A.D.C.	ISMAEL ARTUR DA COSTA ROCHA	3395

ROCHA, L.E.B.	LUIZA EMANUELLE BAIA ROCHA	2987
ROCHA, M.F.	MARLON FORTES ROCHA	2808
ROCHA, M.F.	MARLON FORTES ROCHA	3115
ROCHA, M.F.	MARLON FORTES ROCHA	3271
ROCHA, P.	PETER ROCHA	3080
ROCHA, V.	VICTOR ROCHA	2670
ROCHA, V.D.M.	VICTOR DE MELO ROCHA	2674
ROCHA, V.D.M.	VICTOR DE MELO ROCHA	2692
ROCHA, V.G.	VANDERSON GERALDO ROCHA	2762
ROCHA, V.G.	VANDERSON GERALDO ROCHA	2854
ROCHA, V.G.	VANDERSON GERALDO ROCHA	2855
ROCHA, V.G.	VANDERSON GERALDO ROCHA	3040
ROCHA, V.G.	VANDERSON GERALDO ROCHA	3221
ROCHA, V.G.	VANDERSON GERALDO ROCHA	3226
ROCHA, V.G.	VANDERSON GERALDO ROCHA	3236
ROCHA, V.G.	VANDERSON GERALDO ROCHA	3410
ROCHA, V.N.	VINICIUS NOVAES ROCHA	2834
ROCHA, V.N.	VINICIUS NOVAES ROCHA	3458
ROCHA, V.P.C.	VINICIUS PINTO COSTA ROCHA	3183
ROCHAEL, N.C.	NATALIA CADAXO ROCHAEL	2856
ROCHAEL, N.C.	NATALIA CADAXO ROCHAEL	2872
ROCHAEL, N.C.	NATALIA CADAXO ROCHAEL	2999
ROCHAEL, N.C.	NATALIA CADAXO ROCHAEL	3049
ROCHAEL, N.C.	NATALIA C. ROCHAEL	2898
ROCHAEL, N.C.	NATALIA C. ROCHAEL	3009
RODA, V.M.D.P.	VINICIUS MORAES DE PAIVA RODA	3074
RODOLPHI, C.M.	CINTHIA MAGALHÃES RODOLPHI	2743
RODOLPHI, C.M.	CINTHIA MAGALHÃES RODOLPHI	3050
RODOLPHI, C.M.	CINTHIA MAGALHÃES RODOLPHI	3355
RODOLPHO, J.M.D.A.	JOICE MARGARETH DE ALMEIDA RODOLPHO	3255
RODOLPHO, J.M.D.A.	JOICE MARGARETH DE ALMEIDA RODOLPHO	3297
RODOVALHO, V.D.R.	VINICIUS DE REZENDE RODOVALHO	2798
RODOVALHO, V.D.R.	VINICIUS DE REZENDE RODOVALHO	2803
RODRIGUES JUNIOR, L.C.	LUIZ CARLOS RODRIGUES JUNIOR	3117
RODRIGUES JUNIOR, L.C.	LUIZ CARLOS RODRIGUES JUNIOR	3254
RODRIGUES JUNIOR, L.C.	LUIZ CARLOS RODRIGUES JUNIOR	3368
RODRIGUES, A.C.D.O.	ANNA CLARA DE OLIVEIRA RODRIGUES	2987
RODRIGUES, A.F.F.	ANDRÉ FELLIPE FREITAS RODRIGUES	2736
RODRIGUES, A.F.F.	ANDRÉ FELLIPE FREITAS RODRIGUES	2749
RODRIGUES, A.F.F.	ANDRÉ FELLIPE FREITAS RODRIGUES	2934
RODRIGUES, A.J.R.	ANA JÚLIA ROCHA RODRIGUES	3448
RODRIGUES, B.C.M.	BEATRIZ C. M. RODRIGUES	2632
RODRIGUES, B.C.M.	BEATRIZ C. M. RODRIGUES	2633
RODRIGUES, B.C.M.	BEATRIZ C. M. RODRIGUES	3347
RODRIGUES, B.C.M.	BEATRIZ C. M. RODRIGUES	3509
RODRIGUES, C.	CARLA RODRIGUES	2816
RODRIGUES, C.M.	CÍNTIA MARIA RODRIGUES	3165
RODRIGUES, D.A.S.	DANIELLE APARECIDA SOUSA RODRIGUES	2818
RODRIGUES, D.A.S.	DANIELLE APARECIDA SOUSA RODRIGUES	2941
RODRIGUES, D.A.S.	DANIELLE APARECIDA SOUSA RODRIGUES	2963
RODRIGUES, D.A.S.	DANIELLE APARECIDA SOUSA RODRIGUES	2977
RODRIGUES, D.A.S.	DANIELLE APARECIDA SOUSA RODRIGUES	3085
RODRIGUES, D.G.M.	DÉBORA GOMES MARINS RODRIGUES	3085
RODRIGUES, F.C.	FABIANA CRISTINA RODRIGUES	3491
RODRIGUES, F.C.	FABIANA CRISTINA RODRIGUES	3494
RODRIGUES, F.R.	FABIANA RESENDE RODRIGUES	3392
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2734
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2735
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2741

RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2878
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2889
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2938
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2944
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2966
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2967
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	2982
RODRIGUES, G.M.B.	GABRIELE MANAMY BABA RODRIGUES	3076
RODRIGUES, G.O.L.	GISELE OLINTO LIBANIO RODRIGUES	2751
RODRIGUES, H.G.	HOSANA GOMES RODRIGUES	2783
RODRIGUES, H.G.	HOSANA GOMES RODRIGUES	2803
RODRIGUES, H.G.	HOSANA GOMES RODRIGUES	2817
RODRIGUES, H.G.	HOSANA GOMES RODRIGUES	2869
RODRIGUES, J.H.S.	JOSÉ HENRIQUE SILVA RODRIGUES	2834
RODRIGUES, J.V.G.	JÚLIA VALLE GONÇALVES RODRIGUES	2648
RODRIGUES, J.V.G.	JÚLIA VALLE GONÇALVES RODRIGUES	3347
RODRIGUES, L.S.	LUCAS SILVA RODRIGUES	3103
RODRIGUES, L.S.	LUCAS SILVA RODRIGUES	3190
RODRIGUES, M.C.D.O.	MARIA CAROLINA DE OLIVEIRA RODRIGUES	3120
RODRIGUES, M.M.J.	MÁRCIA MARIA JARDIM RODRIGUES	3481
RODRIGUES, M.P.M.D.	MIGUEL PIRES MEDEIROS DINIZ RODRIGUES	3411
RODRIGUES, N.C.	NAJARA CAVALCANTE RODRIGUES	2838
RODRIGUES, N.C.	NAJARA CAVALCANTE RODRIGUES	3164
RODRIGUES, P.F.	PATRICK FERNANDES RODRIGUES	2843
RODRIGUES, P.H.	PEDRO HENRIQUE RODRIGUES	3032
RODRIGUES, R.R.	RODRIGO RIBEIRO RODRIGUES	3409
RODRIGUES, T.	TIAGO RODRIGUES	3039
RODRIGUES, T.S.	TAMARA SILVA RODRIGUES	3130
RODRIGUES, T.S.	TAMARA SILVA RODRIGUES	3185
RODRIGUES, T.S.	TAMARA SILVA RODRIGUES	3369
RODRIGUES, T.S.	TAMARA SILVA RODRIGUES	3387
RODRIGUES, T.S.	TAMARA SILVA RODRIGUES	3391
RODRIGUES, V.F.	VANESSA FERNANDES RODRIGUES	2766
RODRIGUES, V.F.	VANESSA FERNANDES RODRIGUES	3180
RODRIGUES, V.F.	VANESSA FERNANDES RODRIGUES	3438
RODRIGUES-DA-SILVA, R.N.	RODRIGO NUNES RODRIGUES-DA-SILVA	2743
RODRIGUES-DA-SILVA, R.N.	RODRIGO NUNES RODRIGUES-DA-SILVA	2893
RODRIGUES-DA-SILVA, R.N.	RODRIGO NUNES RODRIGUES-DA-SILVA	3356
RODRIGUES-DA-SILVA, R.N.	RODRIGO NUNES RODRIGUES-DA-SILVA	3431
RODRIGUEZ, J.	JAIR RODRIGUEZ	2903
ROEDERER, M.	MARIO ROEDERER	2715
ROGINSKI, A.C.	ANA CRISTINA ROGINSKI	3315
ROJAS, J.E.U.	JOSE EDUARDO ULLOA ROJAS	2928
ROLIANO, G.G.	GABRIELA GONÇALVES ROLIANO	2880
ROLLA, V.C.	VALERIA CAVALCANTI ROLLA	3505
ROMA, E.H.	ERIC HENRIQUE ROMA	3359
ROMANO, J.V.P.	JOÃO VICTOR PAIVA ROMANO	3343
ROMANO, T.	THAÍS ROMANO	3060
ROMÃO, P.R.T.	PEDRO ROOSEVELT TORRES ROMÃO	2826
ROMÃO, P.R.T.	PEDRO ROOSEVELT TORRES ROMÃO	3254
ROMÃO, P.R.T.	PEDRO ROOSEVELT TORRES ROMÃO	3408
ROMÃO, P.R.T.	PEDRO ROOSEVELT TORRES ROMÃO	3449
ROMERO, D.B.	DANIEL BROWN ROMERO	2844
ROMERO, O.B.	OSCAR BRUNA ROMERO	3449
RONDINELLI, A.	AMANDA RONDINELLI	3029
ROPELLE, E.R.	EDUARDO ROCHETE ROPELLE	2869
ROPER, P.	PERI ROPER	3066
ROQUE, M.C.	MARJORIE C. ROQUE	3276
RÓS, F.A.	FELIPE AUGUSTO RÓS	3410

ROSA, B.G.	BRUNA GENNARI ROSA	2761
ROSA, B.G.	BRUNA GENNARI ROSA	2771
ROSA, B.G.	BRUNA GENNARI ROSA	2791
ROSA, B.G.	BRUNA GENNARI ROSA	2793
ROSA, D.L.S.O.	DALILA LAPINHA SILVA OLIVEIRA ROSA	3281
ROSA, D.S.	DANIELA SANTORO ROSA	2740
ROSA, D.S.	DANIELA SANTORO ROSA	3149
ROSA, D.S.	DANIELA SANTORO ROSA	3211
ROSA, L.C.	LORENN CARVALHO ROSA	3154
ROSA, L.C.	LORENN CARVALHO ROSA	3243
ROSA, L.C.	LORENN CARVALHO ROSA	3249
ROSA, L.C.	LORENN CARVALHO ROSA	3411
ROSA, M.J.G.	MARIA JULIA GRANERO ROSA	2632
ROSA, M.J.G.	MARIA JULIA GRANERO ROSA	2633
ROSA, M.J.G.	MARIA JULIA GRANERO ROSA	3347
ROSA, N.P.	NATÁLIA PINHEIRO ROSA	2673
ROSA, P.C.S.	PALOMA CRISITINA SILVA ROSA	2939
ROSA, P.S.	PATRÍCIA SAMMARCO ROSA	3401
ROSA, S.G.	SAMARA GUIMARÃES ROSA	3086
ROSA, S.G.	SAMARA GUIMARÃES ROSA	3166
ROSA, S.G.	SAMARA GUIMARÃES ROSA	3169
ROSA, T.L.S.A.	THABATTA LEAL SILVEIRA ANDREZO ROSA	3228
ROSA, T.L.S.A.	THABATTA LEAL SILVEIRA ANDREZO ROSA	3466
ROSA, U.G.C.	UALTER GUILHERME CIPRIANO ROSA	3188
ROSADAS, L.A.D.S.	LEONARDO AZEVEDO DA SILVA ROSADAS	3000
ROSAS, E.C.	ELAINE CRUZ ROSAS	3043
ROSSA JR, C.	CARLOS ROSSA JR	3013
ROSSA JR, C.	CARLOS ROSSA JR	3299
ROSSA JR, C.	CARLOS ROSSA JR	3301
ROSSETTI, R.	RAFAELA ROSSETTI	3016
ROSSI-BERGMANN, B.	BARTIRA ROSSI-BERGMANN	2823
ROSSI-BERGMANN, B.	BARTIRA ROSSI-BERGMANN	3343
ROSSO, S.D.	SEBASTIÁN DEL ROSSO	2840
ROZINI, S.V.	STEPHANE VICENTE ROZINI	3049
ROZINI, S.V.	STEPHANE VICENTE ROZINI	3479
RUAS, T.C.B.	TERESA CRISTINA BRITO RUAS	3521
RUAS, Y.F.	YGOR FAGUNDES RUAS	3010
RUBIO, G.G.	GEMA GONZALEZ RUBIO	3027
RUIZ, D.E.V.	DAN ERICK VIVAS RUIZ	2981
RUIZ, D.F.	DANIEL FERNANDEZ RUIZ	2989
RUSSO, E.M.D.S.	ELISA MARIA DE SOUSA RUSSO	2974
RUSSO, M.	MOMTCHILLO RUSSO	2835
RUSSO, M.	MOMTCHILLO RUSSO	3140
RUSSO, R.D.C.	REMO DE CASTRO RUSSO	2675
RUSSOMANO, F.B.	FABIO BASTOS RUSSOMANO	3058
SÁ, J.D.O.	JAMILE DE OLIVEIRA SÁ	2733
SÁ, M.V.B.O.	MARCUS V B OLIVEIRA SÁ	2833
SAAD, M.J.A.	MARIO JOSÉ ABDALLA SAAD	2882
SABINO, A.D.P.	ADRIANO DE PAULA SABINO	2640
SABINO, A.D.P.	ADRIANO DE PAULA SABINO	2691
SABINO, A.D.P.	ADRIANO DE PAULA SABINO	2693
SABINO, E.C.	ESTER CERDEIRA SABINO	2972
SABOIA-VAHIA, L.	LEONARDO SABOIA-VAHIA	3009
SACRAMENTO, I.R.F.	ISABELA REBECA FERNANDES SACRAMENTO	3365
SAGIORATO, R.N.	ROBERTA NICOLLI SAGIORATO	2783
SAGIORATO, R.N.	ROBERTA NICOLLI SAGIORATO	2817
SAKURAGUI, C.M.	CÁSSIA MONIKA SAKURAGUI	3005
SALAZAR, N.	NATALIA SALAZAR	3276
SALES, G.A.M.	GUILHERME AUGUSTO MACHADO SALES	2931

SALGADO, B.F.	BEATRIZ FERREIRA SALGADO	2954
SALGADO, C.L.	CAIO LOUREIRO SALGADO	2645
SALGADO, C.L.	CAIO LOUREIRO SALGADO	2889
SALGADO, C.L.	CAIO LOUREIRO SALGADO	2935
SALGADO, C.L.	CAIO LOUREIRO SALGADO	2944
SALIBA, A.M.	ALESSANDRA MATTOS SALIBA	3390
SALLES, P.G.O.	PAULO GUILHERME O. SALLES	2635
SALLES, P.P.Z.	PRISCILA PINI ZENATTI SALLES	2751
SALLES, P.P.Z.	PRISCILA PINI ZENATTI SALLES	2947
SALLES, P.P.Z.	PRISCILA PINI ZENATTI SALLES	2949
SALLES, P.P.Z.	PRISCILA PINI ZENATTI SALLES	2973
SALM, L.	LILIAN SALM	2853
SALOMÃO, L.F.C.	LUCAS FERNANDES CHAMHUM SALOMÃO	2674
SALOMÃO, R.	REINALDO SALOMÃO	2899
SALOMÃO, R.	REINALDO SALOMÃO	2923
SALVADOR, M.R.	MAIARA RODRIGUES SALVADOR	3051
SALVALÁGIO, B.R.	BEATRIZ RUGILA SALVALÁGIO	2932
SALVI, M.	MARISA SALVI	2701
SAMPAIO, L.A.	LORRANY ALMEIDA SAMPAIO	2810
SAMPAIO, L.A.	LORRANY ALMEIDA SAMPAIO	3136
SAMPAIO, M.S.D.A.	MARLON SILVA DE ALMEIDA SAMPAIO	2758
SANCHES, R.C.D.O.	RODRIGO CÉSAR DE OLIVEIRA SANCHES	2651
SANCHES, R.C.D.O.	RODRIGO CÉSAR DE OLIVEIRA SANCHES	2828
SANCHEZ, E.M.R.	EDUARDO MILTON RAMOS SANCHEZ	3281
SANCHEZ, E.O.F.	ELADIO OSWALDO FLORES SANCHEZ	2981
SANCHEZ, E.O.F.	ELADIO OSWALDO FLORES SANCHEZ	2993
SANCHEZ, L.R.B.	LUCAS RANGEL BERTOLINI SANCHEZ	2809
SANDIM, V.	VANESSA SANDIM	3329
SANGENIS, L.H.	LUIZ HENRIQUE SANGENIS	3404
SANGENIS, L.H.C.	LUIZ HENRIQUE CONDE SANGENIS	3359
SANT'ANNA, L.S.D.S.	LOUISY SANCHES DOS SANTOS SANT'ANNA	2874
SANT'ANNA, L.S.D.S.	LOUISY SANCHES DOS SANTOS SANT'ANNA	2911
SANTANA, B.	BRUNNA SANTANA	3510
SANTANA, D.P.	DAVI PEREIRA SANTANA	2972
SANTANA, H.M.	HALLISON MOTA SANTANA	2971
SANTANA, H.M.	HALLISON MOTA SANTANA	2983
SANTANA, J.B.	JORDANA BATISTA SANTANA	3370
SANTANA, J.B.	JORDANA BATISTA SANTANA	3432
SANTANA, L.F.D.	LÍVIA FERNANDA DIAS SANTANA	2698
SANTANA, L.F.D.	LÍVIA FERNANDA DIAS SANTANA	2699
SANTANA, L.F.D.	LÍVIA FERNANDA DIAS SANTANA	2702
SANTANA, L.F.D.	LÍVIA FERNANDA DIAS SANTANA	3470
SANTANA, M.M.F.	MAYARA MICHELLE FERREIRA SANTANA	3427
SANTANA, R.C.	RAÍSSA COUTO SANTANA	3481
SANTANA, R.D.C.	RODRIGO DE CARVALHO SANTANA	3264
SANTANA, R.P.D.J.	REBECA PINHEIRO DE JESUS SANTANA	3341
SANTANA-GONÇALVES, M.	MAYNARA SANTANA-GONÇALVES	3120
SANT'ANNA, L.D.O.	LINCOLN DE OLIVEIRA SANT'ANNA	2874
SANT'ANNA, L.D.O.	LINCOLN DE OLIVEIRA SANT'ANNA	2911
SANTAREM, G.N.	GUSTAVO NOLASCO SANTAREM	3426
SANTIAGO, H.D.C.	HELTON DA COSTA SANTIAGO	2634
SANTIAGO, H.D.C.	HELTON DA COSTA SANTIAGO	2640
SANTIAGO, M.S.I.D.C.	MARTHA SUELY ITAPARICA DE CARVALHO SANTIAGO	3312
SANTIAGO-CARVALHO, I.	IGOR SANTIAGO-CARVALHO	2935
SANTIAGO-CARVALHO, I.	IGOR SANTIAGO-CARVALHO	3325
SANTO, C.C.D.E.	CAMILA COUTO DO ESPÍRITO SANTO	2930
SANTO, K.P.D.S.	KAUAN PINHEIRO DA SILVA SANTO	2825
SANTO, K.P.D.S.	KAUAN PINHEIRO DA SILVA SANTO	2830
SANTO, K.P.D.S.	KAUAN PINHEIRO DA SILVA SANTO	2859

SANTO, K.P.D.S.	KAUAN PINHEIRO DA SILVA SANTO	2881
SANTOS, A.L.D.C.	ANA LUIZA DE CASTRO SANTOS	2686
SANTOS, A.L.D.C.	ANA LUIZA DE CASTRO SANTOS	2988
SANTOS, A.O.M.	ALBERTO OLIVEIRA MOREIRA SANTOS	2871
SANTOS, A.O.M.	ALBERTO OLIVEIRA MOREIRA SANTOS	2887
SANTOS, A.O.M.	ALBERTO OLIVEIRA MOREIRA SANTOS	2910
SANTOS, A.O.M.	ALBERTO OLIVEIRA MOREIRA SANTOS	3025
SANTOS, Á.R.C.	ÁQUILA RODRIGUES COSTA SANTOS	2931
SANTOS, B.M.D.A.	BÁRBARA MARIA DE AMORIM SANTOS	2657
SANTOS, B.M.D.A.	BÁRBARA MARIA DE AMORIM SANTOS	2675
SANTOS, B.M.D.A.	BÁRBARA MARIA DE AMORIM SANTOS	2685
SANTOS, B.M.D.A.	BÁRBARA MARIA DE AMORIM SANTOS	3434
SANTOS, B.M.D.A.	BÁRBARA MARIA DE AMORIM SANTOS	3451
SANTOS, C.M.R.	CLARICE MONTEIRO RODRIGUES SANTOS	2963
SANTOS, C.P.	CAMILA PIMENTEL SANTOS	3098
SANTOS, C.P.	CAMILA PIMENTEL SANTOS	3231
SANTOS, C.S.P.D.O.	CLARA SERDEIRA PERGENTINO DE OLIVEIRA SANTOS	2825
SANTOS, C.S.P.D.O.	CLARA SERDEIRA PERGENTINO DE OLIVEIRA SANTOS	2830
SANTOS, C.S.P.D.O.	CLARA SERDEIRA PERGENTINO DE OLIVEIRA SANTOS	2859
SANTOS, C.S.P.D.O.	CLARA SERDEIRA PERGENTINO DE OLIVEIRA SANTOS	2864
SANTOS, C.S.P.D.O.	CLARA SERDEIRA PERGENTINO DE OLIVEIRA SANTOS	2888
SANTOS, D.V.	DANIEL VASCONCELOS SANTOS	2715
SANTOS, E.C.	ERIANE CERQUEIRA SANTOS	2931
SANTOS, E.T.	EDUARDO TARAZONA SANTOS	2833
SANTOS, F.D.S.	FRANCISNAIRA DA SILVA SANTOS	3341
SANTOS, F.G.P.	FERNANDA GARCIA PRAIA SANTOS	2900
SANTOS, F.G.P.	FERNANDA GARCIA PRAIA SANTOS	3414
SANTOS, F.R.D.S.	FELIPE ROCHA DA SILVA SANTOS	2685
SANTOS, F.R.D.S.	FELIPE ROCHA DA SILVA SANTOS	2686
SANTOS, G.D.A.	GISLANE DE ALMEIDA SANTOS	3325
SANTOS, I.D.O.	IGOR DE OLIVEIRA SANTOS	3392
SANTOS, I.F.M.	ISABELLA FERNANDES MARTINS SANTOS	2653
SANTOS, I.F.M.	ISABELLA FERNANDES MARTINS SANTOS	2654
SANTOS, J.D.	JEIMINSON DUARTE SANTOS	3269
SANTOS, J.D.	JULIANE DUARTE SANTOS	2987
SANTOS, J.D.	JULIANE DUARTE SANTOS	3165
SANTOS, J.D.C.	JULIA DA CUNHA SANTOS	3257
SANTOS, J.D.C.	JULIA DA CUNHA SANTOS	3480
SANTOS, J.D.C.	JULIA DA CUNHA SANTOS	3482
SANTOS, J.D.C.	JULIA DA CUNHA SANTOS	3490
SANTOS, J.M.	JÚLIA MORATELLI SANTOS	2897
SANTOS, J.M.	JÚLIA MORATELLI SANTOS	3099
SANTOS, J.M.	JÚLIA MORATELLI SANTOS	3132
SANTOS, J.M.	JÚLIA MORATELLI SANTOS	3138
SANTOS, J.M.	JÚLIA MORATELLI SANTOS	3195
SANTOS, J.M.	JÚLIA MORATELLI SANTOS	3238
SANTOS, J.M.	JÚLIA MORATELLI SANTOS	3241
SANTOS, J.M.	JÚLIA MORATELLI SANTOS	3256
SANTOS, J.S.	JAKELINE SERTORIO SANTOS	3160
SANTOS, J.S.	JAKELINE SERTORIO SANTOS	3168
SANTOS, J.S.	JAKELINE SERTORIO SANTOS	3206
SANTOS, K.P.D.S.	KAUAN PINHEIRO DA SILVA SANTOS	2864
SANTOS, L.D.	LEONARDO DUARTE SANTOS	3435
SANTOS, L.G.P.	LUIZ GUSTAVO PONTES SANTOS	3474
SANTOS, L.L.D.O.	LAURA LIS DE OLIVEIRA SANTOS	2699
SANTOS, L.L.D.O.	LAURA LIS DE OLIVEIRA SANTOS	2701
SANTOS, L.L.D.O.	LAURA LIS DE OLIVEIRA SANTOS	2702
SANTOS, L.L.D.O.	LAURA LIS DE OLIVEIRA SANTOS	3470
SANTOS, L.L.O.	LAURA LIS OLIVEIRA SANTOS	2698

SANTOS, M.	MAYARA SANTOS	2835
SANTOS, M.A.L.	MAELI ANDRESSA LÍRIO SANTOS	2826
SANTOS, M.D.O.	MARCELO DE OLIVEIRA SANTOS	3248
SANTOS, M.D.O.	MARCELO DE OLIVEIRA SANTOS	3331
SANTOS, M.D.O.	MARCELO DE OLIVEIRA SANTOS	3336
SANTOS, M.L.	MILENA LIMA SANTOS	2850
SANTOS, M.L.	MILENA LIMA SANTOS	3366
SANTOS, N.D.S.	NATHALIA DA SILVA SANTOS	2960
SANTOS, R.A.	ROBERT ANDREATA SANTOS	2740
SANTOS, R.A.	ROBERT ANDREATA SANTOS	2939
SANTOS, R.A.	ROBERT ANDREATA SANTOS	3042
SANTOS, R.L.	REINAN LIMA SANTOS	3156
SANTOS, R.M.	RICARDO MARTINS SANTOS	3262
SANTOS, R.T.	RENATA TOURINHO SANTOS	2812
SANTOS, S.D.S.	SARAH DA SILVA SANTOS	3282
SANTOS, S.M.E.	SILVANA MARIA ELÓI SANTOS	2630
SANTOS, S.S.	SIDNEIA SOUSA SANTOS	2899
SANTOS, T.A.P.	THAIZA ALINE PEREIRA SANTOS	2692
SANTOS, V.R.	VICTOR RODRIGUES SANTOS	2676
SANTOS, W.G.S.	WILIAS GREISON SILVA SANTOS	2809
SANTOS, W.G.S.	WILIAS GREISON SILVA SANTOS	2829
SANTOS, W.G.S.	WILIAS GREISON SILVA SANTOS	2832
SANTOS, W.G.S.	WILIAS GREISON SILVA SANTOS	2863
SANTOS, W.G.S.	WILIAS GREISON SILVA SANTOS	2882
SANTOS, W.G.S.	WILIAS GREISON SILVA SANTOS	3034
SANTOS, W.G.S.	WILIAS GREISON SILVA SANTOS	3437
SANTOS, W.G.S.	WILIAS GREISON S. SANTOS	2772
SANTOS-FILHO, N.A.	NORIVAL ALVES SANTOS-FILHO	3163
SARAIVA, E.	ELVIRA SARAIVA	2856
SARAIVA, E.	ELVIRA SARAIVA	2872
SARAIVA, E.	ELVIRA SARAIVA	2898
SARAIVA, E.	ELVIRA SARAIVA	2905
SARAIVA, E.	ELVIRA SARAIVA	2955
SARAIVA, E.	ELVIRA SARAIVA	2999
SARAIVA, E.	ELVIRA SARAIVA	3009
SARAIVA, E.	ELVIRA SARAIVA	3049
SARAIVA, E.	ELVIRA SARAIVA	3278
SARAIVA, E.	ELVIRA SARAIVA	3304
SARAIVA, N.	NADJANIA SARAIVA	2722
SARAIVA, N.	NADJANIA SARAIVA	2726
SARAIVA, R.M.	ROBERTO MAGALHÃES SARAIVA	3359
SARAIVA, R.M.	ROBERTO MAGALHÃES SARAIVA	3404
SARDELI, A.V.	AMANDA VEIGA SARDELI	2866
SARMENTO, I.V.	ISABELA VALIM SARMENTO	3059
SARMENTO, I.V.	ISABELA VALIM SARMENTO	3227
SARMENTO, I.V.	ISABELA VALIM SARMENTO	3409
SARNO, E.N.	EUZENIR NUNES SARNO	3228
SARNO, E.N.	EUZENIR NUNES SARNO	3481
SARTORI, A.A.	ARTHUR ALVES SARTORI	3335
SARTORI, D.C.	DANIELA CARLOS SARTORI	2729
SARTORI, D.C.	DANIELA CARLOS SARTORI	2766
SARTORI, D.C.	DANIELA CARLOS SARTORI	3104
SARTORI, D.C.	DANIELA CARLOS SARTORI	3180
SARTORI, D.C.	DANIELA CARLOS SARTORI	3269
SARTORI, D.C.	DANIELA CARLOS SARTORI	3438
SATER, P.P.	PIETRA PERONDI SATER	3285
SATO, H.I.	HUGO ITARU SATO	2643
SATO, H.I.	HUGO ITARU SATO	2645
SATO, H.I.	HUGO ITARU SATO	2664

SATO, M.N.	MARIA NOTOMI SATO	2730
SATO, M.N.	MARIA NOTOMI SATO	2767
SATO, M.N.	MARIA NOTOMI SATO	3038
SATO, M.N.	MARIA NOTOMI SATO	3137
SATOSKAR, A.R.	ABHAY R. SATOSKAR	3210
SAUD, Z.	ZACK SAUD	2978
SAVI, R.V.	RODRIGO VIEIRA SAVI	3179
SAVI, R.V.	RODRIGO VIEIRA SAVI	3257
SAVIETO, R.M.	ROBERTA MARIA SAVIETO	2777
SAVIO, L.E.B.	LUIZ EDUARDO BAGGIO SAVIO	3306
SAVIO, L.E.B.	LUIZ EDUARDO BAGGIO SAVIO	3393
SAVIO, L.E.B.	LUIZ EDUARDO BAGGIO SAVIO	3467
SAVIO, L.E.B.	LUIZ EDUARDO BAGGIO SAVIO	3491
SAVIO, L.E.B.	LUIZ EDUARDO BAGGIO SAVIO	3494
SAYER, C.	CLAUDIA SAYER	3499
SCARINI, J.F.	JOÃO FIGUEIRA SCARINI	3060
SCARINI, J.F.	JOÃO FIGUEIRA SCARINI	3293
SCARINI, J.F.	JOÃO FIGUEIRA SCARINI	3317
SCHERER, M.M.	MILLENA MACHADO SCHERER	3353
SCHETINO, M.A.A.	MARCO ANTÔNIO ALVES SCHETINO	2987
SCHETTINO, K.D.C.	KARLA DE CARVALHO SCHETTINO	2635
SCHIMIDT, D.	DAYANE SCHIMIDT	2802
SCHINEIDER, M.	MATHIAS SCHINEIDER	3219
SCHIPPER, L.D.L.	LUCAS DE LIMA SCHIPPER	2826
SCHMIDT FILHO, J.	JAYR SCHMIDT FILHO	3029
SCHMIDT, F.	FABIAN SCHMIDT	3413
SCHMITZ, G.J.H.	GABRIELA JUSTAMANTE HANDEL SCHMITZ	2776
SCHNEIDER, F.S.	FRANCISCO SANTOS SCHNEIDER	2683
SCHOENFELT, K.	KELLY SCHOENFELT	2882
SCHUCH, V.	VIVIANE SCHUCH	3149
SCHUCK, A.B.	ATHOS BRANDIELLI SCHUCK	3488
SCHWARTZ, S.	SAMUEL SCHWARTZ	3066
SCOPEL, K.K.G.	KEZIA KATIANI GORZA SCOPEL	2834
SCOPEL, K.K.G.	KEZIA KATIANI GORZA SCOPEL	3342
SCOTTON, C.V.	CAROLINE VENDRAME SCOTTON	2821
SCOTTON, C.V.	CAROLINE VENDRAME SCOTTON	2896
SEABRA, M.V.C.	MARIANA VIANNA CHAVES SEABRA	2947
SEADI, R.D.	RAFAEL DUTRA SEADI	3117
SEADI, R.D.	RAFAEL DUTRA SEADI	3368
SEBBA, M.E.C.	MARIA EDUARDA C. SEBBA	2927
SEGATI, A.F.	ANA FLAVIA SEGATI	3011
SEGATI, A.F.	ANA FLAVIA SEGATI	3061
SEGHETO, J.A.	JÚLIA ALMEIDA SEGHETO	3081
SEGHETO, J.A.	JÚLIA ALMEIDA SEGHETO	3082
SEGHETO, J.A.	JÚLIA ALMEIDA SEGHETO	3102
SEGHETO, J.A.	JÚLIA ALMEIDA SEGHETO	3162
SEGURA, G.G.	GABRIELA GONZALEZ SEGURA	3270
SEGURO, F.S.	FERNANDA SALLES SEGURO	2854
SEIBERLICK, O.S.G.	OTTO SAMUEL GONÇALVES SEIBERLICK	3287
SEIBERLICK, T.D.S.O.	THAÍS DE SOUZA OLIVEIRA SEIBERLICK	3287
SEIBLITZ, G.C.	GIULIA CAMINHA SEIBLITZ	3329
SEID, V.E.	VICTOR EDMOND SEID	3060
SEITO, L.N.	LEONARDO NOBORU SEITO	3043
SEIXAS, E.R.	ELOY RAMOS SEIXAS	3032
SENA, N.C.	NATALE CARDOSO SENA	2850
SENA, N.C.	NÁTALE CARDOSO SENA	3182
SENA, N.C.	NÁTALE CARDOSO SENA	3366
SEQUEIRA, Y.D.C.	YASMIN DA CUNHA SEQUEIRA	3390
SEQUERRA, E.B.	EDUARDO BOUTH SEQUERRA	3068

SEREZANI, C.H.	CARLOS HENRIQUE SEREZANI	3125
SEREZANI, C.H.	CARLOS HENRIQUE SEREZANI	3459
SEREZANI, H.	HENRIQUE SEREZANI	2783
SETTE, A.	ALESSANDRO SETTE	2719
SETÚBAL, S.D.S.	SULAMITA DA SILVA SETÚBAL	2971
SETÚBAL, S.D.S.	SULAMITA DA SILVA SETÚBAL	2983
SEVERINO, M.B.	MATHEUS BRANDEMARTSE SEVERINO	2755
SEVERINO, M.B.	MATHEUS BRANDEMARTSE SEVERINO	3088
SEVERINO, M.B.	MATHEUS BRANDEMARTSE SEVERINO	3097
SEVERINO, M.B.	MATHEUS BRANDEMARTSE SEVERINO	3170
SFORCIN, J.M.	JOSÉ MAURÍCIO SFORCIN	3335
SHEPHERD, B.	BRYAN SHEPHERD	3362
SHER, A.	ALAN SHER	2715
SHIGAEFF, N.	NÁDIA SHIGAEFF	3247
SHIGAEFF, N.	NÁDIA SHIGAEFF	3273
SHIGAEFF, N.	NÁDIA SHIGAEFF	3456
SHIKU, H.	HIROSHI SHIKU	2861
SHIKU, H.	HIROSHI SHIKU	2870
SHIKU, H.	HIROSHI SHIKU	2959
SHIKU, H.	HIROSHI SHIKU	3016
SILVA FILHO, A.P.E.	ALVARO PACHECO E SILVA FILHO	2784
SILVA FILHO, A.P.E.	ALVARO PACHECO E SILVA FILHO	3143
SILVA JUNIOR, J.L.	JAIR LIRIO SILVA JUNIOR	3483
SILVA, A.A.	ANDREA ALICE SILVA	2797
SILVA, A.A.	ANDREA ALICE SILVA	2810
SILVA, A.A.	ANDREA ALICE SILVA	2816
SILVA, A.A.	ANDREA ALICE SILVA	2995
SILVA, A.A.	ANDREA ALICE SILVA	3006
SILVA, A.A.	ANDREA ALICE SILVA	3136
SILVA, A.A.	ANDREA ALICE SILVA	3245
SILVA, A.C.F.	ANA CAROLINA FONSECA SILVA	2834
SILVA, A.E.N.E.	ANA ELISA NOLASCO E SILVA	2640
SILVA, A.L.C.A.E.	ANA LAURA CLEMPI ALMEIDA E SILVA	3454
SILVA, A.L.C.A.E.	ANA LAURA CLEMPI ALMEIDA E SILVA	3486
SILVA, A.L.C.A.E.	ANA LAURA CLEMPI ALMEIDA E SILVA	3493
SILVA, A.L.C.A.E.	ANA LAURA CLEMPI ALMEIDA E SILVA	3495
SILVA, A.L.C.A.E.	ANA LAURA CLEMPI ALMEIDA E SILVA	3497
SILVA, A.L.C.A.E.	ANA LAURA CLEMPI ALMEIDA E SILVA	3516
SILVA, A.Q.	ADRIANO QUEIROZ SILVA	2946
SILVA, A.R.	ADRIANA RIBEIRO SILVA	3439
SILVA, A.S.	ANDRÉIA S SILVA	2833
SILVA, B.M.	BRUNO MOREIRA SILVA	2932
SILVA, B.M.M.	BÁRBARA MÁRCIA MARINHO SILVA	2634
SILVA, B.M.S.	BRUNA MANUELLA SOUZA SILVA	3088
SILVA, B.M.S.	BRUNA MANUELLA SOUZA SILVA	3369
SILVA, B.S.D.A.	BRUNA SPOLADOR DE ALENCAR SILVA	2866
SILVA, C.A.A.	CARLLA ASSIS ARAUJO SILVA	3106
SILVA, C.A.A.	CARLLA ASSIS ARAUJO SILVA	3239
SILVA, C.A.C.	CAROLINA ALVES COSTA SILVA	3508
SILVA, C.E.A.	CONCEIÇÃO ELIDIANNE ANÍBAL SILVA	3022
SILVA, C.G.	CAMILA GRIGOLO SILVA	2643
SILVA, D.B.D.F.	DANIEL BENÍCIO DE FREITAS SILVA	3336
SILVA, E.D.S.	EDSON DOS SANTOS SILVA	3437
SILVA, E.N.	EVANDRO NEVES SILVA	2962
SILVA, E.N.	EVANDRO NEVES SILVA	2965
SILVA, E.N.	EVANDRO NEVES SILVA	3003
SILVA, F.M.D.C.E.	FLAVIA MARCIA DE CASTRO E SILVA	2801
SILVA, F.M.D.C.E.	FLAVIA MARCIA DE CASTRO E SILVA	2813
SILVA, F.M.D.C.E.	FLAVIA MARCIA DE CASTRO E SILVA	2822

SILVA, F.M.D.C.E.	FLAVIA MARCIA DE CASTRO E SILVA	2874
SILVA, F.M.D.C.E.	FLAVIA MARCIA DE CASTRO E SILVA	2911
SILVA, F.M.D.C.E.	FLAVIA MARCIA DE CASTRO E SILVA	3116
SILVA, G.	GABRIELA SILVA	2987
SILVA, G.A.D.S.	GUILHERME ANTONIO DE SOUZA SILVA	2984
SILVA, G.A.D.S.	GUILHERME ANTONIO DE SOUZA SILVA	3073
SILVA, G.A.D.S.	GUILHERME ANTONIO DE SOUZA SILVA	3149
SILVA, G.C.	GABRIELE CAROLINE SILVA	3513
SILVA, G.D.A.	GIULIA D'AVILA ALVES SILVA	2672
SILVA, G.D.A.	GIULIA D'AVILA ALVES SILVA	2865
SILVA, G.D.S.	GUILHERME DE SOUZA SILVA	3473
SILVA, G.G.	GUSTAVO GONÇALVES SILVA	3133
SILVA, G.G.	GUSTAVO GONÇALVES SILVA	3293
SILVA, G.R.	GLEICE RAYANNE SILVA	3341
SILVA, G.T.	GABRIELA TERRA SILVA	3454
SILVA, G.T.	GABRIELA TERRA SILVA	3493
SILVA, H.D.S.	HATILLA DOS SANTOS SILVA	3312
SILVA, H.M.	HERNANDEZ MOURA SILVA	2783
SILVA, I.M.E.	ISABELLA MOREIRA E SILVA	2677
SILVA, I.M.E.	ISABELLA MOREIRA E SILVA	2682
SILVA, J.	JULIANA SILVA	3125
SILVA, J.D.C.	JULIANA DA COSTA SILVA	3264
SILVA, J.G.D.	JOAO GABRYEL DANTAS SILVA	2899
SILVA, J.K.A.D.O.	JEFERSON KELVIN ALVES DE OLIVEIRA SILVA	3422
SILVA, J.K.A.D.O.	JEFERSON KELVIN ALVES DE OLIVEIRA SILVA	3500
SILVA, J.L.S.	JORGE LUÍS SANTOS SILVA	2900
SILVA, J.L.S.	JORGE LUÍS SANTOS SILVA	3414
SILVA, J.M.	JULIETH MURILLO SILVA	3229
SILVA, J.M.D.N.	JEFFERSON MANOEL DO NASCIMENTO SILVA	3188
SILVA, J.S.	JOÃO SANTANA SILVA	2983
SILVA, J.S.	JOÃO SANTANA SILVA	3079
SILVA, J.S.	JOÃO SANTANA SILVA	3158
SILVA, J.S.	JOÃO SANTANA SILVA	3264
SILVA, J.S.	JOÃO SANTANA SILVA	3369
SILVA, J.S.	JOÃO SANTANA SILVA	3372
SILVA, J.S.	JOÃO SANTANA SILVA	3375
SILVA, J.S.	JOÃO SANTANA SILVA	3376
SILVA, J.S.	JOÃO SANTANA SILVA	3428
SILVA, K.L.	KARINA LANI SILVA	2927
SILVA, K.L.M.	KLEBER LUÍS MARQUES SILVA	3312
SILVA, K.O.	KAYLAINY OLIVEIRA SILVA	3165
SILVA, L.A.M.T.	LAYS ADRIANNE MENDONCA TRAJANO SILVA	3472
SILVA, L.D.R.	LAYARA DA ROCHA SILVA	3111
SILVA, L.F.L.	LINCON FELIPE LIMA SILVA	2809
SILVA, L.F.L.	LINCON FELIPE LIMA SILVA	2831
SILVA, L.F.L.	LINCON FELIPE LIMA SILVA	3004
SILVA, L.F.L.	LINCON FELIPE LIMA SILVA	3365
SILVA, L.F.L.	LINCON FELIPE LIMA SILVA	3380
SILVA, L.F.L.	LINCON FELIPE LIMA SILVA	3487
SILVA, L.F.S.E.	LUIZ FELIPE SOUZA E SILVA	3074
SILVA, L.M.	LUCIANA MARIA SILVA	2981
SILVA, L.M.	LUCIANA MARIA SILVA	2993
SILVA, L.M.	LUÍSA MENEZES SILVA	3076
SILVA, L.P.	LUCIANO PAULINO SILVA	2906
SILVA, L.P.	LUCIANO PAULINO SILVA	3100
SILVA, L.P.	LUCIANO PAULINO SILVA	3450
SILVA, L.P.	LUIZA PINHEIRO SILVA	2698
SILVA, L.P.	LUIZA PINHEIRO SILVA	2699
SILVA, L.P.	LUIZA PINHEIRO SILVA	2701

SILVA, L.P.	LUIZA PINHEIRO SILVA	2702
SILVA, L.P.	LUIZA PINHEIRO SILVA	3470
SILVA, L.R.B.	LEONARDO RIBEIRO BATISTA SILVA	3135
SILVA, L.R.B.	LEONARDO RIBEIRO BATISTA SILVA	3142
SILVA, L.R.B.	LEONARDO RIBEIRO BATISTA SILVA	3153
SILVA, L.R.B.	LEONARDO RIBEIRO BATISTA SILVA	3332
SILVA, L.R.B.	LEONARDO RIBEIRO BATISTA SILVA	3385
SILVA, L.R.B.	LEONARDO RIBEIRO BATISTA SILVA	3446
SILVA, L.S.	LETÍCIA SOLDATI SILVA	2686
SILVA, L.S.	LETÍCIA SOLDATI SILVA	2988
SILVA, M.A.	MAGAIVER ANDRADE SILVA	3506
SILVA, M.C.	MARIA CAROLINE SILVA	3118
SILVA, M.C.S.	MAYARA CRISTINI SEBASTIÃO SILVA	2758
SILVA, M.D.	MURILLO DUARTE SILVA	3381
SILVA, M.D.O.	MARIANGELA DE OLIVEIRA SILVA	2740
SILVA, M.D.O.	MARIANGELA DE OLIVEIRA SILVA	3007
SILVA, M.D.O.	MARIANGELA DE OLIVEIRA SILVA	3149
SILVA, M.D.S.	MILENA DANIELA SOUZA SILVA	2971
SILVA, M.D.S.	MILENA DANIELA SOUZA SILVA	2983
SILVA, M.E.F.	MARIA EDUARDA FREITAS SILVA	2657
SILVA, M.E.F.	MARIA EDUARDA FREITAS SILVA	2675
SILVA, M.E.F.	MARIA EDUARDA FREITAS SILVA	2685
SILVA, M.E.F.	MARIA EDUARDA FREITAS SILVA	3316
SILVA, M.E.F.	MARIA EDUARDA FREITAS SILVA	3434
SILVA, M.E.F.	MARIA EDUARDA FREITAS SILVA	3451
SILVA, M.F.	MARIANA FERREIRA SILVA	2689
SILVA, M.F.A.S.E.	MARIA FERNANDA ALVES SOUZA E SILVA	2642
SILVA, M.F.D.S.C.	MARIA FERNANDA DE SOUZA COSTA SILVA	3043
SILVA, M.F.D.S.C.	MARIA FERNANDA DE SOUZA COSTA SILVA	3063
SILVA, M.F.D.S.C.	MARIA FERNANDA DE SOUZA COSTA SILVA	3257
SILVA, M.F.D.S.C.	MARIA FERNANDA DE SOUZA COSTA SILVA	3429
SILVA, M.J.D.B.E.	MILTON JOSÉ DE BARROS E SILVA	2842
SILVA, M.J.D.B.E.	MILTON JOSÉ DE BARROS E SILVA	3309
SILVA, M.M.P.E.	MATHEUS MENESES PEREIRA E SILVA	3282
SILVA, M.V.D.M.	MARCUS VINICIUS DE MATTOS SILVA	3095
SILVA, N.B.	NATÁLIA BRUNETTI SILVA	2978
SILVA, N.C.	NATHÁLIA COSTA SILVA	3474
SILVA, P.A.D.C.	PATRÍCIA ALVES DE CASTRO SILVA	2808
SILVA, P.A.D.C.	PATRÍCIA ALVES DE CASTRO SILVA	3080
SILVA, R.D.J.	RAIANI DE JESUS SILVA	3165
SILVA, R.S.F.	RENNAN SALES FERREIRA SILVA	2813
SILVA, R.S.F.	RENNAN SALES FERREIRA SILVA	2822
SILVA, R.S.F.	RENNAN SALES FERREIRA SILVA	3116
SILVA, R.V.D.S.	RENATA VIEIRA DE SOUSA SILVA	3352
SILVA, S.D.A.	SILVANA DE ARAUJO SILVA	2972
SILVA, S.D.A.	SILVANA DE ARAUJO SILVA	2976
SILVA, S.D.A.	SILVANA DE ARAUJO SILVA	3234
SILVA, S.L.S.	SOFIA LÍRIO SANTOS SILVA	3156
SILVA, S.L.S.	SOFIA LÍRIO SANTOS SILVA	3160
SILVA, S.L.S.	SOFIA LÍRIO SANTOS SILVA	3168
SILVA, S.L.S.	SOFIA LÍRIO SANTOS SILVA	3206
SILVA, T.C.	THIAGO CERQUEIRA SILVA	3318
SILVA, T.G.D.S.E.	THAIANY GOULART DE SOUZA E SILVA	2976
SILVA, T.L.	TAMIRES LOPES SILVA	2928
SILVA, V.O.	VALÉRIA OLIVEIRA SILVA	3002
SILVA, V.R.D.P.E.	VITÓRIA REGINA DE PAULA E SILVA	3421
SILVA, Y.P.	YGOR PARLADORE SILVA	2931
SILVA1, R.C.	RAPHAEL CHAGAS SILVA1	3182
SILVA1, R.C.	RAPHAEL CHAGAS SILVA1	3366

SILVEIRA, A.C.A.	ANNA CLARA AZEVEDO SILVEIRA	2695
SILVEIRA, A.C.A.	ANNA CLARA AZEVEDO SILVEIRA	2696
SILVEIRA, A.C.A.	ANNA CLARA AZEVEDO SILVEIRA	2697
SILVEIRA, A.C.A.	ANNA CLARA AZEVEDO SILVEIRA	2700
SILVEIRA, A.C.A.	ANNA CLARA AZEVEDO SILVEIRA	2703
SILVEIRA, A.C.A.	ANNA CLARA AZEVEDO SILVEIRA	2704
SILVEIRA, C.G.T.	CÁSSIA G. T. SILVEIRA	3362
SILVEIRA, C.G.T.	CÁSSIA G. T. SILVEIRA	3476
SILVEIRA, G.D.B.	GUSTAVO DE BEM SILVEIRA	3118
SILVEIRA, G.F.	GUILHERME F SILVEIRA	3259
SILVEIRA, J.R.	JOÃO RAPHAEL SILVEIRA	3022
SILVEIRA-NUNES, G.	GABRIELA SILVEIRA-NUNES	2643
SILVEIRA-NUNES, G.	GABRIELA SILVEIRA-NUNES	2664
SILVERIO, Y.A.D.C.	YURI ALEXANDRE DE CARVALHO SILVERIO	2918
SILVESTRE, R.N.	RENATA NACASAKI SILVESTRE	2802
SILVESTRINI, M.M.A.	MARINA MALHEIROS ARAUJO SILVESTRINI	2635
SILVESTRINI, M.M.A.	MARINA MALHEIROS ARAUJO SILVESTRINI	2650
SIMÕES, A.C.B.L.	ALINE CRISTINA BRANDO LIMA SIMÕES	3521
SIMÕES, M.M.	MARINA MESQUITA SIMÕES	2763
SIMÕES, M.M.	MARINA MESQUITA SIMÕES	2764
SINCERO, T.C.M.	THAÍS CRISTINE MARQUES SINCERO	3449
SINHORIN, V.D.G.	VALÉRIA DORNELLES GINDRI SINHORIN	2737
SINHORIN, V.D.G.	VALÉRIA DORNELLES GINDRI SINHORIN	3322
SINHORIN, V.D.G.	VALÉRIA DORNELLES GINDRI SINHORIN	3398
SINHORIN, V.D.G.	VALÉRIA DORNELLES GINDRI SINHORIN	3448
SIQUEIRA, M.M.	MARILDA MENDONÇA SIQUEIRA	3187
SIZO, F.D.J.P.D.F.	FABRÍCIA DE JESUS PAIVA DA FONSECA SIZO	3408
SKALINSKI, L.M.	LACITA MENEZES SKALINSKI	3312
SOARES, A.M.	ANDREIMAR MARTINS SOARES	2971
SOARES, A.M.	ANDREIMAR MARTINS SOARES	2983
SOARES, B.F.S.	BERNARDO FREIRE STARLING SOARES	2643
SOARES, B.F.S.	BERNARDO FREIRE STARLING SOARES	2645
SOARES, B.F.S.	BERNARDO FREIRE STARLING SOARES	2683
SOARES, D.D.M.	DENIS DE MELO SOARES	3370
SOARES, G.O.	GABRIELLE OLIVEIRA SOARES	3450
SOARES, H.A.D.S.	HELLEN ANASTÁCIA DA SILVA SOARES	3376
SOARES, I.D.S.	IRENE DA SILVA SOARES	2950
SOARES, I.D.S.	IRENE DA SILVA SOARES	3007
SOARES, I.F.	ISABELA FERREIRA SOARES	2743
SOARES, I.F.	ISABELA FERREIRA SOARES	2893
SOARES, I.F.	ISABELA FERREIRA SOARES	3050
SOARES, I.F.	ISABELA FERREIRA SOARES	3355
SOARES, M.A.	MARIANA ALVES SOARES	2904
SOARES, M.A.	MARIANA ALVES SOARES	3306
SOARES, M.B.P.	MILENA BOTELHO PEREIRA SOARES	3183
SOARES, M.B.P.	MILENA BOTELHO PEREIRA SOARES	3341
SOARES, S.M.A.	SARA MALAGUTI ANDRADE SOARES	3287
SOARES, S.M.A.	SARA MALAGUTI ANDRADE SOARES	3330
SOARES, S.M.A.	SARA MALAGUTI ANDRADE SOARES	3336
SOARES, V.C.	VINICIUS CARDOSO SOARES	3426
SOARES, V.C.	VINICIUS CARDOSO SOARES	3480
SOARES, V.C.	VINICIUS CARDOSO SOARES	3482
SOARES, V.C.	VINICIUS CARDOSO SOARES	3490
SOBRINHO, M.C.F.	MARIA CLARA FORTES SOBRINHO	2979
SODER, S.	STEPHAN SODER	3289
SODER, S.	STEPHAN SODER	3353
SODER, S.	STEPHAN SODER	3488
SODERO, A.C.R.	ANA CAROLINA RENNÓ SODERO	3523
SODRE, S.	STEFANIE SODRE	3267

SONCINI, T.	THAISE SONCINI	3449
SORGI, C.A.	CARLOS ARTERIO SORGI	2691
SORGI, C.A.	CARLOS ARTERIO SORGI	2861
SORGI, C.A.	CARLOS ARTERIO SORGI	3357
SORGI, C.A.	CARLOS ARTERIO SORGI	3381
SORGI, C.A.	CARLOS ARTERIO SORGI	3412
SORGI, C.A.	CARLOS ARTERIO SORGI	3449
SORGI, C.A.	CARLOS ARTERIO SORGI	3468
SOUSA, A.M.	ARIANE MARTINS SOUSA	2874
SOUSA, A.M.	ARIANE MARTINS SOUSA	2911
SOUSA, D.P.	DANIEL PEREIRA SOUSA	3137
SOUSA, G.C.C.	GIULIA CHIARA CAMILO SOUSA	3383
SOUSA, I.G.	ISABEL GARCIA SOUSA	3144
SOUSA, I.G.	ISABEL GARCIA SOUSA	3242
SOUSA, I.G.	ISABEL GARCIA SOUSA	3424
SOUSA, J.E.A.	JENNIFER EMILY ANUNCIAÇÃO SOUSA	2850
SOUSA, J.E.A.	JENNIFER EMILY ANUNCIAÇÃO SOUSA	3182
SOUSA, J.E.A.	JENNIFER EMILY ANUNCIAÇÃO SOUSA	3197
SOUSA, J.E.A.	JENNIFER EMILY ANUNCIAÇÃO SOUSA	3366
SOUSA, M.B.	MÔNICA BRAGANÇA SOUSA	2899
SOUSA, M.B.	MÔNICA BRAGANÇA SOUSA	2923
SOUSA-SILVA, M.	MARCELO SOUSA-SILVA	3453
SOUZA, C.S.	CLÁUDIA SILVA SOUZA	2917
SOUZA, C.S.M.	CAROLINA SPELING MENDES SOUZA	3118
SOUZA, I.D.F.	ILVA DE FÁTIMA SOUZA	2709
SOUZA, I.D.F.	ILVA DE FÁTIMA SOUZA	2718
SOUZA, J.L.F.M.	JADE LIZ FERREIRA MENDES SOUZA	3197
SOUZA, J.L.F.M.	JADE LIZ FERREIRA MENDES SOUZA	3291
SOUZA, J.L.N.	JORGE LUCAS NASCIMENTO SOUZA	2675
SOUZA, K.B.D.S.	KAMILLA BATISTA DA SILVA SOUZA	3089
SOUZA, K.B.D.S.	KAMILLA BATISTA DA SILVA SOUZA	3091
SOUZA, K.B.D.S.	KAMILLA BATISTA DA SILVA SOUZA	3348
SOUZA, L.	LUIZA SOUZA	3090
SOUZA, L.M.	LORENA MIRANDA SOUZA	3182
SOUZA, L.M.	LORENA MIRANDA SOUZA	3197
SOUZA, L.M.	LORENA MIRANDA SOUZA	3366
SOUZA, L.R.	LÍVIA RINALDI SOUZA	3000
SOUZA, L.R.	LÍVIA RINALDI SOUZA	3064
SOUZA, L.R.	LÍVIA RINALDI SOUZA	3247
SOUZA, M.G.D.S.	MICHAELLE GERALDA DOS SANTOS SOUZA	2987
SOUZA, O.F.	OLIVIA FONSECA SOUZA	2789
SOUZA, O.F.	OLIVIA FONSECA SOUZA	3419
SOUZA, P.E.D.A.	PAOLA EDUARDA DE ALMEIDA SOUZA	2906
SOUZA, P.E.D.A.	PAOLA EDUARDA DE ALMEIDA SOUZA	3096
SOUZA, P.E.D.A.	PAOLA EDUARDA DE ALMEIDA SOUZA	3100
SOUZA, R.	RAFAEL SOUZA	2645
SOUZA, R.D.C.	REBEKA DA CONCEIÇÃO SOUZA	3405
SOUZA, R.S.	ROBERTA S. SOUZA	2833
SOUZA, T.D.S.	TAMYRIS DOS SANTOS SOUZA	3257
SOUZA, T.D.S.	TAMYRIS DOS SANTOS SOUZA	3279
SOUZA, T.D.S.	TAMYRIS DOS SANTOS SOUZA	3439
SOUZA, T.M.L.	THIAGO M. LOPES SOUZA	2686
SOUZA, T.M.L.	THIAGO M. LOPES SOUZA	3479
SOUZA, T.M.L.	THIAGO M. L. SOUZA	3426
SOUZA, T.M.L.	THIAGO MORENO LOPES SOUZA	3482
SOUZA, T.M.L.E.	THIAGO MORENO LOPES E SOUZA	3490
SOUZA, W.C.D.	WANDERSON CAWAN DOURADO SOUZA	3128
SOVERAL, L.	LUCAS SOVERAL	3449
SPARWASSER, T.D.	TIM DOMINIK SPARWASSER	2750

SPECKHORT, D.	DEMÉTRIO SPECKHORT	2984
SPECKHORT, D.	DEMÉTRIO SPECKHORT	3073
SPEGLICH, C.	CARLOS SPEGLICH	3297
SPERANDIO, A.C.F.	ANA CAROLINA FERREIRA SPERANDIO	3051
SPEROTTO, M.G.	MARIANA GAZZONI SPEROTTO	3154
SPONTON, C.H.G.	CARLOS HENRIQUE GROSSI SPONTON	3365
STANCIOLI, E.F.B.	EDEL FIGUEIREDO BARBOSA STANCIOLI	2690
STANTON, R.J.	RICHARD J STANTON	2978
STAROBINAS, N.	NANCY STAROBINAS	2894
STAROBINAS, N.	NANCY STAROBINAS	3225
STEFFKE, E.	EMILY STEFFKE	2929
STEIMBACH, J.V.	JOÃO VITOR STEIMBACH	3048
STEIMBACH, J.V.	JOÃO VITOR STEIMBACH	3135
STEIN, R.T.	RENATO TETELBOM STEIN	3321
STEIN, R.T.	RENATO TETELBOM STEIN	3455
STEINER, A.A.	ALEXANDRE A. STEINER	3416
STOLL, C.R.	CARLY R STOLL	2935
STRAUSS, G.D.F.	GIOVANA DA FONSECA STRAUSS	3354
STRUYF, S.	SOFIE STRUYF	2846
STUDART, I.C.	IGOR CABRAL STUDART	3127
STUDART, I.C.	IGOR CABRAL STUDART	3144
STUDART, I.C.	IGOR CABRAL STUDART	3242
STUDART, I.C.	IGOR CABRAL STUDART	3424
SUAREZ, E.R.	ELOAH RABELLO SUAREZ	2821
SUAREZ, E.R.	ELOAH RABELLO SUAREZ	2896
SUAREZ, E.R.	ELOAH RABELLO SUAREZ	3039
SUAREZ, E.R.	ELOAH RABELLO SUAREZ	3290
SUBRAMANIAN, V.	VEEDAMALI SUBRAMANIAN	3161
SUFFYS, P.N.	PHILIP NOEL SUFFYS	2986
SUGIYAMA, F.H.C.	FABRÍCIA HELOISA CAVICCHIOLI SUGIYAMA	2867
SUGIYAMA, F.H.C.	FABRÍCIA HELOISA CAVICCHIOLI SUGIYAMA	3018
SULCZEWSKI, F.B.	FERNANDO BANDEIRA SULCZEWSKI	2950
SUMAIA, P.A.	PIRES A SUMAIA	2655
SUMIDA, T.M.O.	TELMA MIYUKI OSHIRO SUMIDA	2769
SUMIDA, T.M.O.	TELMA MIYUKI OSHIRO SUMIDA	2776
SUSZTAK, K.	KATALIN SUSZTAK	3506
SUZUKAWA, A.A.	ANDREIA AKEMI SUZUKAWA	2860
SUZUKI, C.I.	CAROLINE ISHIHAMA SUZUKI	3472
SYED, W.A.P.	WASIM ALUÍSIO PRATES SYED	3131
TACONELLI, C.	CESAR TACONELLI	2972
TADA, M.S.	MAURO SHUGIRO TADA	2636
TADA, M.S.	MAURO SHUGIRO TADA	2639
TADA, M.S.	MAURO SHUGIRO TADA	2650
TAKETOMI, E.A.	ERNESTO AKIO TAKETOMI	2705
TAKEUTI, V.Y.	VALERIA YUGUE TAKEUTI	3055
TAKEUTI, V.Y.	VALERIA YUGUE TAKEUTI	3308
TAKEUTI, V.Y.	VALERIA YUGUE TAKEUTI	3361
TAKIYA, C.M.	CHRISTINA MAEDA TAKIYA	3393
TAKIYA, C.M.	CHRISTINA MAEDA TAKIYA	3465
TAMARINDO, G.H.	GUILHERME HENRIQUE TAMARINDO	2733
TAMASHIRO, E.	EDWIN TAMASHIRO	3468
TAMBOURGI, D.V.	DENISE V. TAMBOURGI	3319
TAN, L.B.	LUIS BATISTA TAN	2942
TANURI, A.	AMILCAR TANURI	2941
TANURI, A.	AMILCAR TANURI	2963
TANURI, A.	AMILCAR TANURI	2977
TAPAJÓZ, R.C.D.S.	RAFAEL CARRACENA DE SOUZA TAPAJÓZ	3047
TARRAGÔ, A.M.	ANDRÉA MONTEIRO TARRAGÔ	3395
TASCA, M.G.	MANUELA GONÇALVES TASCA	3521

TASHIMA, A.K.	ALEXANDRE KEIJI TASHIMA	2923
TASMO, N.R.A.	NATALIA RECARDO AMORIM TASMO	2919
TASMO, N.R.A.	NATALIA RECARDO AMORIM TASMO	3328
TASMO, N.R.A.	NATALIA RECARDO AMORIM TASMO	3400
TATIANE, .	TATIANE	2655
TAVARES, E.A.	ERIC ARRIVABENE TAVARES	3409
TAVARES, N.C.	NAIARA CLEMENTE TAVARES	2653
TAVARES, N.C.	NAIARA CLEMENTE TAVARES	2654
TAVARES, N.M.	NATALIA MACHADO TAVARES	2775
TAVARES, N.M.	NATALIA MACHADO TAVARES	2833
TAVARES, N.M.	NATALIA MACHADO TAVARES	3075
TAVARES, N.M.	NATALIA MACHADO TAVARES	3125
TAVARES, N.M.	NATALIA MACHADO TAVARES	3156
TAVARES, N.M.	NATALIA MACHADO TAVARES	3318
TAVARES, N.M.	NATALIA MACHADO TAVARES	3520
TAVONI, T.M.	THAUANY MARTINS TAVONI	2809
TAVONI, T.M.	THAUANY MARTINS TAVONI	2863
TÁVORA, B.D.C.L.F.	BIANCA DE CARVALHO LINS FERNANDES TÁVORA	3225
TEIXEIRA, B.D.A.	BÁRBARA DE ALMEIDA TEIXEIRA	2968
TEIXEIRA, B.T.	BRENO TERRA TEIXEIRA	2876
TEIXEIRA, C.A.	CLARA ANDRADE TEIXEIRA	3124
TEIXEIRA, C.A.	CLARA ANDRADE TEIXEIRA	3139
TEIXEIRA, C.A.	CLARA ANDRADE TEIXEIRA	3223
TEIXEIRA, C.W.	CAIO WILKER TEIXEIRA	2670
TEIXEIRA, C.W.	CAIO WILKER TEIXEIRA	2692
TEIXEIRA, C.W.	CAIO WILKER TEIXEIRA	2714
TEIXEIRA, D.C.	DANIELA CUNHA TEIXEIRA	2685
TEIXEIRA, F.R.	FELIPE ROBERTI TEIXEIRA	3255
TEIXEIRA, G.A.	GABRIEL ANTUNES TEIXEIRA	3086
TEIXEIRA, G.A.	GABRIEL ANTUNES TEIXEIRA	3166
TEIXEIRA, G.A.	GABRIEL ANTUNES TEIXEIRA	3169
TEIXEIRA, G.A.P.B.	GERLINDE AGATE PLATAIS BRASIL TEIXEIRA	2933
TEIXEIRA, H.C.	HENRIQUE COUTO TEIXEIRA	3087
TEIXEIRA, H.M.P.	HELENA MARIANA PITANGUEIRA TEIXEIRA	3291
TEIXEIRA, J.	JOSÉ TEIXEIRA	2866
TEIXEIRA, L.É.A.	LORRANNA ÉVELLYN ALVES TEIXEIRA	3051
TEIXEIRA, M.D.G.L.C.	MARIA DA GLÓRIA LIMA CRUZ TEIXEIRA	3312
TEIXEIRA, M.L.D.C.M.	MARIANA LOPES DA CUNHA MENOSSI TEIXEIRA	3252
TEIXEIRA, M.L.D.C.M.	MARIANA LOPES DA CUNHA MENOSSI TEIXEIRA	3487
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2657
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2675
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2685
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2686
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2699
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2701
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2702
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2846
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2847
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	2988
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	3316
TEIXEIRA, M.M.	MAURO MARTINS TEIXEIRA	3451
TEIXEIRA, M.P.	MARIANA PIRES TEIXEIRA	2758
TEIXEIRA, N.W.	NATÁLIA WNUK TEIXEIRA	2685
TEIXEIRA, S.L.	SAMUEL LUIZ TEIXEIRA	2701
TEIXEIRA, S.L.	SAMUEL LUIZ TEIXEIRA	2702
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	2633
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	2643
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	2645
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	2651

TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	2655
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	2664
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	2672
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	2865
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	3276
TEIXEIRA, S.M.R.	SANTUZA MARIA RIBEIRO TEIXEIRA	3509
TEIXEIRA, S.M.R.	SANTUZA M. R. TEIXEIRA	2641
TEIXEIRA, T.L.	THAISE LARA TEIXEIRA	2697
TELLES, E.	EMILIO TELLES	3429
TEMEROZO, J.	JAIRÓ TEMEROZO	2872
TEMEROZO, J.	JAIRÓ TEMEROZO	2898
TEMEROZO, J.	JAIRÓ TEMEROZO	2907
TEMEROZO, J.	JAIRÓ TEMEROZO	3009
TEMEROZO, J.	JAIRÓ TEMEROZO	3049
TEMEROZO, J.	JAIRÓ TEMEROZO	3393
TEMEROZO, J.	JAIRÓ TEMEROZO	3494
TEMEROZO, J.R.	JAIRÓ RAMOS TEMEROZO	2930
TEÓFILO, F.B.S.	FRANCISCO BRENO SILVA TEÓFILO	2831
TERNETTE, N.	NICOLA TERNETTE	3033
TERRA, E.	ESTER TERRA	3055
TERRA, E.	ESTER TERRA	3279
TERRA, E.	ESTER TERRA	3308
TERRA, E.	ESTER TERRA	3310
TERRA, E.	ESTER TERRA	3361
TERREL, D.L.A.	DANIEL LEONARDO ALZAMORA TERREL	2808
TERREL, D.L.A.	DANIEL LEONARDO ALZAMORA TERREL	3128
TERREL, D.L.A.	DANIEL LEONARDO ALZAMORA TERREL	3224
TERREL, D.L.A.	DANIEL LEONARDO ALZAMORA TERREL	3271
TEZZA, L.C.	LUCIANNA CORRÊA TEZZA	3383
THEISEN, M.	MICHAEL THEISEN	2893
THIMÓTEO, R.R.C.	RACHELL RAMALHO CORREIA THIMÓTEO	2891
THIMÓTEO, R.R.C.	RACHELL RAMALHO CORREIA THIMÓTEO	3005
THIMÓTEO, R.R.C.	RACHELL RAMALHO CORREIA THIMÓTEO	3017
THOMAS, P.G.	PAUL G. THOMAS	3413
THOMATIELI-SANTOS, R.V.	RONALDO VAGNER THOMATIELI-SANTOS	2932
THOMAZELLA, M.V.	MATEUS VAILANT THOMAZELLA	3362
THOMAZELLA, M.V.	MATEUS VAILANT THOMAZELLA	3476
THOMÉ, B.D.C.	BEATRIZ DA COSTA THOMÉ	3476
THORNTON, C.	CHRISTINA THORNTON	2853
TIRAPELLE, M.C.	MARIANE CARIATI TIRAPELLE	2802
TIYO, B.T.	BRUNA TIAKI TIYO	2776
TODESCHINI, A.R.	ADRIANE REGINA TODESCHINI	2724
TODESCHINI, A.R.	ADRIANE REGINA TODESCHINI	3238
TODESCHINI, A.R.	ADRIANE REGINA TODESCHINI	3463
TOLLER-KAWAHISA, J.E.	JULIANA ESCHER TOLLER-KAWAHISA	3487
TOMA, B.T.	BIANCA TIEMI TOMA	2861
TOMÉ, F.D.	FERNANDA DIAS TOMÉ	3342
TOQUETÃO, M.V.L.	MARCOS VINÍCIUS LEMES TOQUETÃO	3362
TOQUETÃO, M.V.L.	MARCOS VINÍCIUS LEMES TOQUETÃO	3476
TORO, D.M.	DIANA MOTA TORO	3468
TORRECILHAS, A.C.T.	ANA CLAUDIA TROCOLI TORRECILHAS	2635
TORRECILHAS, A.C.T.	ANA CLAUDIA TROCOLI TORRECILHAS	2722
TORRECILHAS, A.C.T.	ANA CLAUDIA TROCOLI TORRECILHAS	2726
TORRECILHAS, A.C.T.	ANA CLAUDIA TROCOLI TORRECILHAS	2730
TORRECILHAS, A.C.T.	ANA CLAUDIA TROCOLI TORRECILHAS	3286
TORRES, C.M.L.	CAIO MARQUES LANDEIRO TORRES	3210
TORRES, E.J.L.	EDUARDO JOSE LOPES TORRES	2911
TORRES, J.D.A.	JHOAN DAVID AGUILLÓN TORRES	2704
TORRES, J.D.A.	JHOAN DAVID AGUILLÓN TORRES	2706

TORRES, J.D.A.	JHOAN DAVID AGUILLÓN TORRES	2707
TORRES, J.P.M.	JOÃO PAULO MACHADO TORRES	3471
TORRES, L.	LÍCIA TORRES	2643
TORRES, L.	LÍCIA TORRES	2645
TORRES, L.	LÍCIA TORRES	2656
TORRES, L.	LÍCIA TORRES	2664
TORRES, L.	LÍCIA TORRES	2673
TORRES, L.	LÍCIA TORRES	2677
TORRES, L.	LÍCIA TORRES	2682
TORRES, L.	LÍCIA TORRES	2684
TORRES, L.	LÍCIA TORRES	3067
TORRES, L.A.G.	LIBARDO ANDRÉS GONZÁLEZ TORRES	2709
TORRES, L.A.G.	LIBARDO ANDRÉS GONZÁLEZ TORRES	2718
TORRES, L.M.	LETÍCIA M. TORRES	2666
TORRES, T.A.	THAIS ABDALA TORRES	2638
TORRES, T.A.	THAIS ABDALA TORRES	2649
TORRES, V.J.	VICTOR J. TORRES	3244
TOSCANO, E.C.D.B.	ELIANA CRISTINA DE BRITO TOSCANO	3247
TOSCANO, E.C.D.B.	ELIANA CRISTINA DE BRITO TOSCANO	3273
TOSCANO, E.C.D.B.	ELIANA CRISTINA DE BRITO TOSCANO	3456
TOTINO, P.R.R.	PAULO RENATO RIVAS TOTINO	2743
TOTINO, P.R.R.	PAULO RENATO RIVAS TOTINO	2893
TOTINO, P.R.R.	PAULO RENATO RIVAS TOTINO	2940
TOTINO, P.R.R.	PAULO RENATO RIVAS TOTINO	3050
TOTINO, P.R.R.	PAULO RENATO RIVAS TOTINO	3355
TOTINO, P.R.R.	PAULO RENATO RIVAS TOTINO	3356
TRENTINI, M.M.	MONALISA MARTINS TRENTINI	2791
TREZENA, A.G.	ARYENE GÓES TREZENA	2894
TRINDADE, G.B.	GABRIÉLLY BINDO TRINDADE	3079
TRINDADE, G.B.	GABRIÉLLY BINDO TRINDADE	3264
TRINDADE, P.	PABLO TRINDADE	3523
TRIOZZI, E.	ENZO TRIOZZI	2829
TRIOZZI, E.	ENZO TRIOZZI	3380
TROMBETTA, B.A.	BIANCA A TROMBETTA	2958
TROMBINI, H.	HENRIQUE TROMBINI	3186
TSCHOEKE, D.	DIOGO TSCHOEKE	3508
TSUJI, M.	MORIYA TSUJI	2692
TUBARÃO, L.N.	LUCIANA NEVES TUBARÃO	2812
TUBARÃO, L.N.	LUCIANA NEVES TUBARÃO	3154
TUBARÃO, L.N.	LUCIANA NEVES TUBARÃO	3243
TUBARÃO, L.N.	LUCIANA NEVES TUBARÃO	3411
TUCCI, L.F.F.	LUIZ FERNANDO FORTUNATO TUCCI	3357
TUCCI, L.F.F.	LUIZ FERNANDO FORTUNATO TUCCI	3412
TUFIK, S.	SÉRGIO TUFIK	3211
TUMBACO, C.E.R.	CAROLYN ESTEFANY ROSALES TUMBACO	3503
TUPINAMBÁS, U.	UNAÍ TUPINAMBÁS	2643
TUPINAMBÁS, U.	UNAÍ TUPINAMBÁS	2645
TUPINAMBÁS, U.	UNAÍ TUPINAMBÁS	2664
ULLOA, J.E.	JOSE EDUARDO ULLOA	3140
URBANSKI, A.H.	ALYSSON HENRIQUE URBANSKI	3369
USÓN JUNIOR, P.L.S.	PEDRO LUIZ SERRANO USÓN JUNIOR	3060
UZÊDA, L.F.A.	LAÍSIA FALCÃO ALONSO UZÊDA	3341
VALDEZ, A.F.	ALESSANDRO FERNANDES VALDEZ	3484
VALDEZ, A.F.	ALESSANDRO FERNANDES VALDEZ	3090
VALE, A.M.	ANDRÉ MACEDO VALE	2719
VALE, A.M.	ANDRÉ MACEDO VALE	2724
VALE, A.M.	ANDRÉ MACEDO VALE	2818
VALE, A.M.	ANDRÉ MACEDO VALE	2941
VALE, A.M.	ANDRÉ MACEDO VALE	2963

VALE, A.M.	ANDRÉ MACEDO VALE	2977
VALE, A.M.	ANDRÉ MACEDO VALE	3068
VALE, A.M.	ANDRÉ MACEDO VALE	3085
VALE, A.M.	ANDRÉ MACEDO VALE	3107
VALE, A.M.	ANDRÉ MACEDO VALE	3178
VALE, A.M.	ANDRÉ MACEDO VALE	3502
VALE, L.B.	LARA BARBOSA VALE	2769
VALENÇA, L.D.S.	LUCIANA DOS SANTOS VALENÇA	2925
VALENTIM, C.	CAROLINE VALENTIM	3201
VALENTIM, C.	CAROLINE VALENTIM	3202
VALERA, F.C.P.	FABIANA CARDOSO PEREIRA VALERA	3468
VALERIANO, J.D.P.	JESSICA DO PRADO VALERIANO	3043
VALERIANO, J.D.P.	JESSICA DO PRADO VALERIANO	3063
VALERIO, R.R.	RAFAELA RODRIGUES VALERIO	2876
VALERIO, R.R.	RAFAELA RODRIGUES VALERIO	2931
VALIATE, B.	BRUNO VALIATE	2663
VALLE, G.P.C.	GIULIA PEREIRA COSTA VALLE	3087
VANDENABEELE, P.	PETER VANDENABEELE	3115
VANZAN, D.F.	DANIEL FIGUEIREDO VANZAN	3109
VARGAS, F.R.	FERNANDO REGLA VARGAS	3184
VARGAS, F.R.	FERNANDO REGLA VARGAS	3249
VARGA-WEISZ, P.	PATRICK VARGA-WEISZ	3220
VARGES, V.C.	VITOR COSTA VARGES	3417
VASCONCELOS, L.R.S.	LUYDSON R S VASCONCELOS	2833
VASCONCELOS, Z.	ZILTON VASCONCELOS	3187
VASCONCELOS, Z.	ZILTON VASCONCELOS	3243
VASCONCELOS, Z.	ZILTON VASCONCELOS	3411
VASQUES, V.R.R.	VANESSA ROCHA RIBEIRO VASQUES	2985
VASQUES, V.R.R.	VANESSA ROCHA RIBEIRO VASQUES	3222
VAZ, L.G.	LEONARDO GOMES VAZ	2634
VAZ, L.G.	LEONARDO GOMES VAZ	2828
VEIGA, A.P.R.	ANA PAULA ROCHA VEIGA	2645
VEIGA, M.R.	MARIANA ROMÃO VEIGA	2985
VEIGA, M.R.	MARIANA ROMÃO VEIGA	3222
VELEZ, A.S.M.M.	AFONSO SANTINE MAGALHÃES MESQUITA VELEZ	2912
VELIKKAKAM, T.	TERESIAMA VELIKKAKAM	2695
VELIKKAKAM, T.	TERESIAMA VELIKKAKAM	2696
VELIKKAKAM, T.	TERESIAMA VELIKKAKAM	2697
VELIKKAKAM, T.	TERESIAMA VELIKKAKAM	2700
VELIKKAKAM, T.	TERESIAMA VELIKKAKAM	2703
VELLOSO, V.C.B.	VIDA CARVALHO BOMFIM VELLOSO	3282
VELOSO, V.G.	VALDILÉA GONÇALVES VELOSO	3105
VELOSO, V.G.	VALDILÉA GONÇALVES VELOSO	3240
VELOSO, V.G.	VALDILÉA G. VELOSO	3000
VELOSO, V.G.	VALDILÉA G. VELOSO	3260
VELOSO, V.G.	VALDILÉA G. VELOSO	3362
VELOSO., C.	CRISTINA VELOSO.	3219
VENERANDO, L.A.F.	LETÍCIA APARECIDA FERREIRA VENERANDO	3248
VENERANDO, L.A.F.	LETÍCIA APARECIDA FERREIRA VENERANDO	3280
VENERANDO, L.A.F.	LETÍCIA APARECIDA FERREIRA VENERANDO	3287
VENERANDO, L.A.F.	LETÍCIA APARECIDA FERREIRA VENERANDO	3330
VENERANDO, L.A.F.	LETÍCIA APARECIDA FERREIRA VENERANDO	3331
VENERANDO, L.A.F.	LETÍCIA APARECIDA FERREIRA VENERANDO	3340
VENTURA, L.H.D.A.	LUCAS HANIEL DE ARAÚJO VENTURA	2643
VENTURA, L.H.D.A.	LUCAS HANIEL DE ARAÚJO VENTURA	2645
VENTURA, L.H.D.A.	LUCAS HANIEL DE ARAÚJO VENTURA	2664
VERAS, P.S.T.	PATRICIA SAMPAIO TAVARES VERAS	2775
VERAS, P.S.T.	PATRICIA SAMPAIO TAVARES VERAS	3291
VERAS, P.S.T.	PILAR SAMPAIO TAVARES VERAS	3291

VERÍCIMO, M.A.	MAURÍCIO AFONSO VERÍCIMO	2797
VERONEZES, R.M.	RAUL MOYSES VERONEZES	3344
VERRI, M.P.	MARAISA PALHÃO VERRI	3344
VESSONI, S.C.S.	SANDRA COCCUZZO SAMPAIO VESSONI	2879
VESSONI, S.C.S.	SANDRA COCCUZZO SAMPAIO VESSONI	2902
VIANA, A.S.	ALICE SLOTFELDT VIANA	2814
VIANA, J.C.S.	JULLY CHAYRA SANTOS VIANA	3051
VIANA, K.F.	KELVINSON FERNANDES VIANA	3339
VIANA, L.D.S.	LUCIANA DOS SANTOS VIANA	3323
VIANNA, M.S.	MONICA SANTANA VIANNA	3471
VIANNA, P.H.O.	PEDRO HENRIQUE OLIVEIRA VIANNA	2931
VIANNA, R.A.	RENATA ARTIMOS VIANNA	2742
VICENTE, C.R.	CREUZA RACHEL VICENTE	3227
VICTORA, G.	GABRIEL VICTORA	3107
VICTORA, G.	GABRIEL VICTORA	3178
VICTORA, G.	GABRIEL VICTORA	3413
VICTORA, G.D.	GABRIEL D. VICTORA	3085
VIDAL, A.S.	ANDREY SLADKEVICIUS VIDAL	3286
VIDAL, E.K.D.S.	ERICA KASSIA DE SOUSA VIDAL	3472
VIDAL, J.L.L.	JOÃO LUIZ LUZ VIDAL	2933
VIDAL, V.M.	VINICIUS MENDES VIDAL	2888
VIDAL, V.M.	VINICIUS MENDES VIDAL	2941
VIDAL, V.M.	VINICIUS MENDES VIDAL	2963
VIDAL, V.M.	VINICIUS MENDES VIDAL	2977
VIDAL, V.M.	VINICIUS MENDES VIDAL	3086
VIDAL, V.M.	VINICIUS MENDES VIDAL	3166
VIDAL, V.M.	VINICIUS MENDES VIDAL	3169
VIDAL, V.M.	VINICIUS MENDES VIDAL	3253
VIDAL, V.M.	VINICIUS MENDES VIDAL	3478
VIEGAS JUNIOR, C.	CLAUDIO VIEGAS JUNIOR	3251
VIEIRA JUNIOR, J.C.	JULIANO CUSTODIO VIEIRA JUNIOR	3288
VIEIRA, B.L.M.D.O.	BARBARA LUCIA MORAIS DE OLIVEIRA VIEIRA	2788
VIEIRA, B.L.M.D.O.	BARBARA LUCIA MORAIS DE OLIVEIRA VIEIRA	2915
VIEIRA, B.L.M.D.O.	BARBARA LUCIA MORAIS DE OLIVEIRA VIEIRA	2922
VIEIRA, B.L.M.D.O.	BARBARA LUCIA MORAIS DE OLIVEIRA VIEIRA	2927
VIEIRA, B.L.M.D.O.	BARBARA LUCIA MORAIS DE OLIVEIRA VIEIRA	2942
VIEIRA, B.L.M.D.O.	BARBARA LUCIA MORAIS DE OLIVEIRA VIEIRA	2956
VIEIRA, B.L.M.D.O.	BARBARA LUCIA MORAIS DE OLIVEIRA VIEIRA	2960
VIEIRA, B.L.M.D.O.	BARBARA LUCIA MORAIS DE OLIVEIRA VIEIRA	3150
VIEIRA, E.R.	ETEL ROCHA VIEIRA	2987
VIEIRA, E.R.	ETEL ROCHA VIEIRA	3165
VIEIRA, J.E.	JOAQUIM EDSON VIEIRA	3024
VIEIRA, L.M.	LAYLA MARIA VIEIRA	3108
VIEIRA, L.M.	LAYLA MARIA VIEIRA	3135
VIEIRA, L.M.	LAYLA MARIA VIEIRA	3443
VIEIRA, M.	MAISA VIEIRA	3219
VIEIRA, M.H.F.	MARIA HELOÍSA FERNANDES VIEIRA	3313
VIEIRA, M.S.	MARIANA SOUSA VIEIRA	2640
VIEIRA, O.J.	OSMAR JUNIOR VIEIRA	3503
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	2772
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	2775
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	2809
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	2829
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	2831
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	3034
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	3111
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	3252
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	3298
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	3380

VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAES VIEIRA	3464
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	2832
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	2863
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	2882
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	2920
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	3004
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	3027
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	3365
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	3437
VIEIRA, P.M.M.D.M.	PEDRO MANOEL MENDES DE MORAIS VIEIRA	3487
VIEIRA, R.D.S.	RAQUEL DE SOUZA VIEIRA	2917
VIEIRA, T.S.D.S.	THIAGO SOARES DE SOUZA VIEIRA	3329
VIEIRA, W.W.D.S.	WILLIAM WALLACE DA SILVA VIEIRA	3427
VIGUERA, A.R.	ANA ROSA VIGUERA	3453
VILELA, M.M.D.S.	MARIA MARLUCE DOS SANTOS VILELA	3360
VILLELA, L.M.	LARISSA MELO VILLELA	3064
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	2783
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	2798
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	2843
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	2869
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	2889
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	3067
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	3076
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	3220
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	3269
VINOLO, M.A.R.	MARCO AURÉLIO RAMIREZ VINOLO	3348
VIOLA, J.	JOÃO VIOLA	2922
VIOLA, J.P.D.B.	JOÃO PAULO DE BIASO VIOLA	2788
VIOLA, J.P.D.B.	JOÃO PAULO DE BIASO VIOLA	2915
VIOLA, J.P.D.B.	JOÃO PAULO DE BIASO VIOLA	2927
VIOLA, J.P.D.B.	JOÃO PAULO DE BIASO VIOLA	2942
VIOLA, J.P.D.B.	JOÃO PAULO DE BIASO VIOLA	2956
VIOLA, J.P.D.B.	JOÃO PAULO DE BIASO VIOLA	2960
VIOLA, J.P.D.B.	JOÃO PAULO DE BIASO VIOLA	3150
VISIOLI, F.	FERNANDA VISIOLI	2880
VIVEIROS, D.D.	DIANA DALZY VIVEIROS	2778
VLIET, J.C.V.	JOHANNA CHRISTINE VAN VLIET	2761
VLIET, J.C.V.	JOHANNA CHRISTINE VAN VLIET	2771
VLIET, J.C.V.	JOHANNA CHRISTINE VAN VLIET	2791
VLIET, J.C.V.	JOHANNA CHRISTINE VAN VLIET	2793
VOGELS, C.	CHANTAL VOGELS	2719
VOLPE-FIX, A.R.	ANDRESSA ROEHRIG VOLPE-FIX	2932
WACLAWIAK, I.	INGRID WACLAWIAK	2930
WACLAWIAK, I.	INGRID WACLAWIAK	2955
WACLAWIAK, I.	INGRID WACLAWIAK	3041
WACLAWIAK, I.	INGRID WACLAWIAK	3071
WACLAWIAK, I.	INGRID WACLAWIAK	3106
WACLAWIAK, I.	INGRID WACLAWIAK	3239
WAISMAN, A.	ARI WAISMAN	2766
WAISMAN, A.	ARI WAISMAN	3269
WANDERLEY, J.L.	JOÃO LUIZ WANDERLEY	3469
WANG, Z.	ZIJUN WANG	3007
WATANABE, A.S.A.	ARIPUANÃ SAKURADA ARANHA WATANABE	3336
WATARI, K.	KOSUKE WATARI	2831
WEISSMULLER, G.	GILBERTO WEISSMULLER	3304
WERNECH, M.V.I.	MARIA VICTÓRIA INÁCIO WERNECH	3106
WERNECH, M.V.I.	MARIA VICTÓRIA INÁCIO WERNECH	3239
WERNECK, I.V.T.	ISABELA VITÓRIA TAVARES WERNECK	3144
WERNECK, I.V.T.	ISABELA VITÓRIA TAVARES WERNECK	3242

WERNECK, M.B.D.F.	MIRIAM BIANCHI DE FRONTIN WERNECK	3014
WERNECK, M.B.D.F.	MIRIAM BIANCHI DE FRONTIN WERNECK	3150
WERNECK, M.B.D.F.	MIRIAM BIANCHI DE FRONTIN WERNECK	3274
WERNECK, M.B.D.F.	MIRIAM BIANCHI DE FRONTIN WERNECK	3328
WERNECK, M.B.D.F.	MIRIAM BIANCHI DE FRONTIN WERNECK	3394
WERNECK, M.B.F.	MIRIAM B. F. WERNECK	3426
WEST, J.D.	JORDAN D. WEST	3066
WEYRICH, A.	ANDREW WEYRICH	3247
WEYRICH, A.	ANDREW WEYRICH	3273
WEYRICH, A.	ANDREW WEYRICH	3456
WHITE, E.	EMILY WHITE	2935
WICKI, A.	AMANDA WICKI	2929
WILEMAN, T.	TOM WILEMAN	2808
WILLIAMS, T.C.	THOMAS CHRISTOPHER WILLIAMS	3001
WINTHEREIK, L.	LINE WINTHEREIK	2926
WOODRUFF, T.	TRENT WOODRUFF	3319
WOWK, P.F.	PRYSCILLA FANINI WOWK	2857
WRENGER, C.	CARSTEN WRENGER	2740
WRENGER, C.	CARSTEN WRENGER	2865
WRENGER, C.	CARSTEN WRENGER	3149
WRIGHT, T.D.	THOMAS DUDLEY WRIGHT	3329
XANDER, P.	PATRICIA XANDER	3286
XAVIER JUNIOR, J.T.	JOAQUIM TEIXEIRA XAVIER JUNIOR	3465
XAVIER, A.M.	AMANDA MACHADO XAVIER	2825
XAVIER, A.M.	AMANDA MACHADO XAVIER	2830
XAVIER, A.M.	AMANDA MACHADO XAVIER	2859
XAVIER, A.M.	AMANDA MACHADO XAVIER	2864
XAVIER, F.H.D.C.	FELIPE HENRIQUE DA CUNHA XAVIER	2930
XAVIER, F.H.D.C.	FELIPE HENRIQUE DA CUNHA XAVIER	2931
XAVIER, Í.D.L.D.S.	ÍTALO DE LUCAS DA SILVA XAVIER	3311
XAVIER, L.D.A.	LAURA DO AMARAL XAVIER	2640
XAVIER, T.D.S.	THAÍSSY DOS SANTOS XAVIER	3083
XAVIER, T.D.S.	THAÍSSY DOS SANTOS XAVIER	3272
XIE, W.	WEI XIE	3115
XU, T.T.	TENG TENG XU	3004
YABUUTI, E.M.	ESTHER MIDORI YABUUTI	2803
YAMADA, S.M.	SUEMY MELIM YAMADA	2917
YAMAGUTI-HAYAKAWA, G.G.	GABRIELA GÓES YAMAGUTI-HAYAKAWA	3360
YAMAMOTO, M.M.	MARCIO MASSAO YAMAMOTO	2740
YAMAMOTO, M.M.	MARCIO MASSAO YAMAMOTO	2928
YAMAMOTO, M.M.	MARCIO MASSAO YAMAMOTO	2950
YAMAMOTO, M.M.	MARCIO MASSAO YAMAMOTO	2984
YAMAMOTO, M.M.	MARCIO MASSAO YAMAMOTO	3007
YAMAMOTO, M.M.	MARCIO MASSAO YAMAMOTO	3073
YAMAMOTO, M.M.	MARCIO MASSAO YAMAMOTO	3149
YAMASHIRO, R.	ROSEMEIRE YAMASHIRO	3002
YARIWAKE, V.Y.	VICTOR YUJI YARIWAKE	2909
YARIWAKE, V.Y.	VICTOR YUJI YARIWAKE	2917
YATSUDA, A.P.	ANA PATRÍCIA YATSUDA	3344
YILDIRIM, I.	INCI YILDIRIM	2719
YILMAZ, B.	BAHTIYAR YILMAZ	3086
YING, Y.	YINZHI YING	3161
YUNES, J.A.	JOSÉ ANDRÉS YUNES	2751
ZALESKI, A.	AGNIESZKA ZALESKI	2719
ZAMARREÑO, F.	FERNANDO ZAMARREÑO	2957
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3130
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3157
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3185
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3296

ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3313
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3369
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3384
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3401
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3452
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3465
ZAMBONI, D.S.	DARIO SIMÕES ZAMBONI	3511
ZANA, N.C.T.	NATHALIA CAROLINE TEIXEIRA ZANA	2894
ZANATTA, A.	ANGELA ZANATTA	3455
ZANATTA, J.M.	JONATHAN MIGUEL ZANATTA	3124
ZANATTA, J.M.	JONATHAN MIGUEL ZANATTA	3139
ZANATTA, J.M.	JONATHAN MIGUEL ZANATTA	3223
ZANETTI, L.C.	LARISSA CARDOSO ZANETTI	3472
ZANGIROLAMI, L.A.	LUCAS ALMEIDA ZANGIROLAMI	2742
ZANGROSSI JUNIOR, H.	HELIO ZANGROSSI JUNIOR	3188
ZANI, N.C.	NICHOLAS CAMARGO ZANI	2831
ZANI, N.C.	NICHOLAS CAMARGO ZANI	3004
ZARAMELA, L.S.	LIVIA SOARES ZARAMELA	2724
ZÁRATE-BLADÉS, C.R.	CARLOS RODRIGO ZÁRATE-BLADÉS	3449
ZAULI, R.C.	ROGERIA CRISTINA ZAULI	3286
ZEQUI, S.D.C.	STENIO DE CASSIO ZEQUI	3171
ZHAN, W.	WENDY ZHAN	2853
ZHONG, Y.	YIYA ZHONG	2844
ZHONG, Y.	YI ZHONG	2719
ZIMMERMANN-FRANCO, D.C.	DANIELLE CRISTINA ZIMMERMANN-FRANCO	3203
ZOLLNER, R.D.L.	RICARDO DE LIMA ZOLLNER	2884
ZOLLNER, R.D.L.	RICARDO DE LIMA ZOLLNER	3056
ZONZIN, G.F.	GUILHERME FAHL ZONZIN	3074
ZORDAN, J.S.N.	JULIA STHEFANY NUNES ZORDAN	3227
ZORDAN, J.S.N.	JULIA STHEFANY NUNES ZORDAN	3409
ZUCCHERATO, L.W.	LUCIANA WERNECK ZUCCHERATO	2643
ZUCCHERATO, L.W.	LUCIANA WERNECK ZUCCHERATO	2645
ZUHORN, I.S.	INGE S. ZUHORN	2895
ZULIANI, J.P.	JULIANA PAVAN ZULIANI	2971
ZULIANI, J.P.	JULIANA PAVAN ZULIANI	2983
ZULIANI, J.P.	JULIANA PAVAN ZULIANI	3514
ZUMA, M.C.C.	MARIA CELIA CHAVEZ ZUMA	3174
ZUNIGA, E.	ELINA ZUNIGA	2845

IMMUNO BÚZIOS2025

XLIX Congress of the
Brazilian Society of Immunology (SBI)
October 13th to 17th

SBI

