14th Latin American and Caribbean Congress of Immunology

Buenos Aires, Argentina - November 4-8, 2024

ABSTRACTS BOOK

14th Latin American and Caribbean Congress of Immunology

Buenos Aires, Argentina - November 4-8, 2024

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Latin American and Caribbean Association of Immunology

Argentinean Society of Immunology

Argentinean Association of Clinical Immunologists

November 4-8, 2024
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Welcome message

Dear Members of the Immunology Community,

Join us for the 14th Latin American and Caribbean Immunology Congress (ALACI2024), slated for November 4-8, 2024, in the vibrant city of Buenos Aires, Argentina. Organized by the Latin America and Caribbean Association of Immunology (ALACI), in collaboration with the Argentinean Society of Immunology (SAI) and the Argentinean Association of Clinical Immunologists (AINCA), this event promises an enriching experience.

Under the theme **"Transdisciplinary Innovation to Bring Research Findings Closer to the Clinic"**, the Congress aims to facilitate networking and education by bringing together leading immunologists, healthcare providers, interdisciplinary scientists, clinicians, and aspiring immunologists and students worldwide. Engage in fruitful discussions on recent advances in all fields of Immunology.

The venue, Universidad Católica Argentina, is situated in the cosmopolitan elegance of Puerto Madero, Buenos Aires' premier waterfront district. This area boasts an array of hotels, nightclubs, restaurants, and museums, showcasing modern sophistication amidst picturesque docks. Explore the stunning skyline, featuring gleaming skyscrapers and refurbished warehouses, while indulging in world-class cuisine at trendy waterfront restaurants, savoring Argentina's renowned steak and wine.

Extend your stay to explore the beautiful landscapes of Argentina, including the awe-inspiring Iguazu waterfalls and the majestic Perito Moreno glacier, either before or after the Congress.

We eagerly anticipate your presence in Buenos Aires in November 2024

Sincerely yours,

Emilio Malchiodi, ALACI President
Martín Rumbo, SAI President
Liliana Bezrodnik, AINCA President

1. **Miriam Merad**
Department of Oncological Sciences Tisch Cancer Institute, Hess Center, New York, USA. Targeting myeloid cells in cancer.

2. **Eliane Piaggio**
Institut Curie, PSL University, Inserm U932, Immunity and Cancer, Paris, France. Combined immunotherapies for lowly mutated pediatric tumors, a translational approach.

3. **Adriana Gruppi**
CIBICI-CONICET, Universidad Nacional de Córdoba, Argentina. Protagonists involved in antibody-secreting cells generation and role: breaking the paradigm?

4. **Rita Carsetti**
B Cell Pathophysiology Unit, Immunology Research Area, Bambino Gesù Children's Hospital IRCCS, Rome, Italy. Systemic and mucosal immunity: separate roles and integration strategies.

5. **Gabriel Núñez**
Department of Pathology, University of Michigan (USA). Immune-pathobiont interactions in Crohn's disease.

6. **Alberto Mantovani**
IRCCS Humanitas Research Hospital and Department of Biomedical Sciences, Humanitas University, Milan, Italy. Tumor-associated macrophages in tumor progression and as therapeutic targets.

7. **Ana María Lennon-Duménil**
Institut Curie, INSERM U932, PSL Research University, France. Dendritic cell migration: from fundaments to application.

8. **Sergio Daniel Catz**
Neutrophil-mediated inflammation, from mechanisms to translational approaches. Department of Molecular Medicine, MB215 The Scripps Research Institute, La Jolla, CA, USA.

9. **Andrea Carfi**
Infectious Disease Research, Moderna, Inc., Cambridge, Massachusetts, USA. mRNA technology and Moderna development programs.

10. **Alexis Kalergis**
Instituto Milenio en Inmunología e Inmunoterapia, Pontificia Universidad Católica de Chile, Chile. Vaccine immunity counteracts respiratory virus-mediated suppression of immunological and neurological synapses.

11. **Diane Mathis**
Department of Immunology, Harvard Medical School, Boston, MA, USA. Thymic mimetic cells.

12. **Ricardo Gazzinelli**
Centro de Tecnologia de Vacinas, Universidade Federal de Minas Gerais, Brazil; Instituto René Rachou, Fundação Oswaldo Cruz-Minas, Brazil; Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Brazil. Itaconate impairs immune control of Plasmodium by enhancing mtDNA-mediated PD-L1 expression via IRF3/IRF7 pathway.

13. **María Luisa Alegre**
Department of Medicine, University of Chicago, Chicago, IL, USA. Tregs, T cell hypofunction and transplantation tolerance.

14. **Juan Carlos Zúñiga-Pflücker**
Department of Immunology, University of Toronto; and Biological Sciences, Sunnybrook Research Institute, Toronto, Ontario, Canada. Teaching new tricks to an old thymus.

15. **Christophe Benoist**
Department of Immunology, Harvard Medical School, Boston, MA, USA. Regulating Tregs.

16. **Rudolf Valenta**
Center for Pathophysiology, Infectiology & Immunology, Medical University of Vienna, Austria. A comprehensive approach for molecular diagnosis and vaccination against allergy.

17. **Sebastián Amigorena**
Institut Curie, PSL University, Inserm U932, Immunity and Cancer, Paris, France. New targets for cancer immunotherapy.

18. **Norberto Zwirner**
Instituto de Biología y Medicina Experimental (IBYME-CONICET), Fundación IBYME, Laboratorio de Fisiopatología de la Inmunidad Innata; and Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Universidad de Buenos Aires, Buenos Aires, Argentina. Immunotherapy beyond checkpoints: NKG2D ligands as targets for precision medicine.

19. **Gabriel Rabinovich**
Laboratorio de Glicomedicina, Instituto de Biología y Medicina Experimental (IBYME), CONICET; and Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. The power of glyco-immune-checkpoints: Translating glycan-encoded information into novel therapies.

MAIN SYMPOSIUM 1: ANTI-INFECTIOUS IMMUNITY.

Olivier Neyrolles.
CNRS - Institute of Pharmacology and Structural Biology, France. Neuro-immune interactions in the tuberculosis granuloma.
Andrés Alloatti.
Instituto de Inmunología Clínica y Experimental de Rorsario, Argentina. Antigens of Trypanosoma cruzi: a long journey from dendritic cells to… dendritic cells!
Edécio Cunha-Neto.
Laboratory of Clinical immunology and Allergy, Faculty of Medicine, Sao Paulo University, Brasil. Cytokine-induced mitochondrial dysfunction: a new paradigm and therapeutic target in the pathogenesis of Chagas disease cardiomyopathy.

MAIN SYMPOSIUM 2: TRANSLATIONAL ONCO-IMMUNOLOGY AND IMMUNOTHERAPY.

José Cohen.
Mondor Institute for biomedical research INSERM U955, France. Targeting Treg through TNFR2 in onco-immunology.
Rosendo Luria Pérez.
Unit of Investigative Research on Oncological Diseases, Children's Hospital of Mexico Federico Gomez, Mexico City, Mexico. Live-Attenuated Salmonella enterica is a successful delivery system of peptides and genes for cancer therapy.
Álvaro Lladser.
Laboratory of Immunoncology, Fundación Ciencia & Vida, Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile. Deciphering the T cell networks underlying effective immunity against solid tumors.

MAIN SYMPOSIUM 3: INBORN ERRORS OF IMMUNITY.

Sergio Rozensweig.
Department of Laboratory Medicine, NIH Clinical Center, USA;. Pneumocystis pneumonia (PCP) susceptibility: new genes and mechanisms of disease.
Jacinta Bustamante.
Center for the Study of Primary Immunodeficiencies, Necker Hospital for Sick Children, Paris, France. Human inborn errors of IFN-gamma immunity.
Arnaud Didierlaurent.
Facultè de Medicine, Universitè de Genève, Switzerland. Learnings from studying vaccine response in immunocompromised patients.

MAIN SYMPOSIUM 4: NEUROIMMUNOLOGY AND NEUROINFLAMMATION.

Rodrigo Pacheco.
Funcación Ciencia y Vida, Universidad de San Sebastián, Chile. Developing an immunotherapy for Parkinson's disease: Regulatory T-cells expressing a chimeric antigen receptor specific to nitrated alpha-synuclein.
Sourav Ghosh.
Department of Neurology and Department of Pharmacology, Yale University School of Medicine, United States of America. Microglial receptor function and immunoresilience in Alzheimer's disease.
Rodrigo Naves.
Institute of Biomedical Sciences, Facultad de Medicina, Universidad de Chile, Chile. The Yin and Yang of interferon-gamma in experimental and human Multiple Sclerosis.

MAIN SYMPOSIUM 5: TRANSPLANTATION

Ignacio Anegón.
Nantes University, INSERM 1064-CR2TI, France. CD8 Treg therapy in organ allo transplantation and acute GVHD.
Karina Pino Lagos.
Centro de Investigación e Innovación Biomédica (CiiB). Facultad de Medicina. Universidad de Los Andes, Chile. Participation of FoxP3+ T regulatory cells in a murine skin transplantation model.

MAIN SYMPOSIUM 6: NEW APPROACHES IN INBORN ERRORS OF IMMUNITY

Pere Soler Palacín.
Pediatric Infectious Diseases and Immunodeficiencies Unit, Children's Hospital, Vall d'Hebron Barcelona, Spain. Screening opportunities for Inborn Errors of Immunity: Starting a new era with AI.

MAIN SYMPOSIUM 7:

Belén Almejún.
IQUIBICEN-CONICET- Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina. Molecular diagnostic experience using whole exome sequencing in AINCA patients across the country.

MAIN SYMPOSIUM 8: INNATE IMMUNITY AND INFLAMMATION.

Marco Cassatella.
Department of Medicine, Section of General Pathology, University of Verona, Verona, Italy. Advancing our knowledge of human mature PMN-MDSCs.
Carla Rothlin.
Yale University School of Medicine, New Haven, USA. Decoding the response to cell death in non-resolving inflammation.
Kazuyo Moro.
RIKEN Center for Integrative Medical Sciences (IMS), Kanagawa, and Graduate School of Medicine, Osaka University, Osaka, Japan. Eubiosis caused by appendectomy suppress ulcerative colitis through ILC2.

MAIN SYMPOSIUM 9: HOST-PATHOGEN INTERACTIONS I

David Russell.
Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA. The impact of macrophage diversity on tuberculosis progression.
Marina Palermo.
Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina. Neutralizing antibodies in the intestinal mucosa are essential to control Shiga toxin-producing Escherichia coli (STEC) gastrointestinal infection.
Sergio Costa Oliveira.
Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. Trained innate immunity: BCG-induced protection in infectious diseases and cancer.

MAIN SYMPOSIUM 10: AINCA - AUTOINFLAMMATORY DISORDERS (CLINICAL CASES)

Lucia Tarquini.
Hospital de Niños "Sor María Ludovica" de La Plata, Buenos Aires, Argentina.
Astrid Schellnast
Faure. Hospital de Niños "Sor María Ludovica" de La Plata, Buenos Aires, Argentina.
Gustavo Martin (Argentina).
Centro de Inmunologia Clínica Dra.Liliana Bezrodnik y Equipo-CABA, Argentina.

MAIN SYMPOSIUM 11: REPRODUCTIVE IMMUNOLOGY.

Lachlan Moldenhauer.
Robinson Research Institute and School of Biomedicine, The University of Adelaide, Adelaide, SA, Australia. Metabolic dysfunction and Treg cell lineage instability contributing to immune imbalance in recurrent pregnancy loss.
Rossana Ramhorst.
Instituto de Química Biológica, Facultad de Ciencias Exactas y Naturales-CONICET-UBA, CABA, Argentina. Insights into the immune embryo-endometrium interactions.
Ana Zenclussen.
Helmholtz-Centre for Environmental Research - UFZ GmbH, Leipzig, Germany. Unlocking the womb: deciphering feto-maternal immune dialogue for healthy pregnancy outcomes.

MAIN SYMPOSIUM 12: MUCOSAL IMMUNOLOGY (LAMIG I)

Ana María Caetano Faria.
Departamento de Bioquímica e Imunologia. Universidade Federal de Minas Gerais (UFMG). Brazil. Neuroimmune circuits involved in food allergy to peanuts.
Charles A. Parkos.
Department of Pathology, School of Medicine, University of Michigan, Ann Arbor, Michigan, USA. Regulation of mucosal repair during injury and inflammation.
Marcelo Valdemir de Araujo.
Institute of Biomedical Sciences. Department of Immunology of the University of São Paulo, Brazil. Ketogenic diet modifies lung microbiota composition and impacts tissue-specific immunity.
Glauben Landskron.
Center for Biomedical Research, CIBMED, Facultad de Medicina Universidad Finis Terrae, Chile. The association of an RNA-editing protein in the regulatory and exhausted T cell subsets in the intestine.

MAIN SYMPOSIUM 13: CELLULAR AND MOLECULAR IMMUNOLOGY

Fabiola Osorio.
Facultad de Medicina Universidad de Chile, Chile. Regulation of intestinal Th17 homeostasis by the unfolded protein response IRE1 in myeloid cells.
Burkhard Becher.
Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland. GM-CSF: from growth factor to proinflammatory cytokine and back again.
Karina Bortoluci.
Department of Pharmacology, Federal University of São Paulo, Brazil. How do inflammasomes shape the astrocytes' ability to fight infections.

MAIN SYMPOSIUM 14: AUTOIMMUNITY AND INFLAMMATORY DISEASES

Florencia Rosetti.
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico. PDGFRa protects against autoimmune-mediated tissue injury.
Luisina Onofrio.
CIBICI -CONICET-Facultad de Ciencias Químicas. Universidad Nacional de Córdoba, Argentina. Effect of JAK kinase inhibitors on T cell immune response in patients with rheumatoid arthritis.
Patricia Alejandra Luz Crawford.
Facultad de Medicina, Universidad de los Andes, Santiago, Chile. Mitochondria transfer from mesenchymal stromal cells to memory T-CD4 cells as a potential therapy for inflammatory.

MAIN SYMPOSIUM 15: LEUKOCYTES IN HEALTH AND DISEASE AND NEW STANDARDS IN FLOW CYTOMETRY

Mats W Johansson.
Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, University of Wisconsin, Madison, Wisconsin, USA. Eosinophil integrin activation, adhesion and migration, and associations with disease.
Michael Schnoor.
Department for Molecular Biomedicine, CINVESTAV del IPN, Mexico. Neutrophil recruitment requires endothelial cortactin degradation by neutrophil serine proteases.
Virginia Litwin.
Virginia Litwin. Eurofins Clinical Trial Solutions, Montreal, QC, Canada. New Standards for Clinical and Translational Cytometry.

MAIN SYMPOSIUM 16: VACCINES

Juliana Cassataro.
Instituto de Investigaciones Biotecnológicas - CONICET-Universidad de San Martín, Argentina. Development and clinical evaluation of ARVAC: a variant adapted protein subunit vaccine against SARS-CoV-2.
Jorge Kalil.
Heart Institute (InCor-HC-FMUSP). School of Medicine of the University of São Paulo (FMUSP), Brazil. Development of a intranasal vacine to SARS-CoV-2.

MAIN SYMPOSIUM 17: IMMUNODEFICIENCIES I

Sergio Rosenzweig.
Immunology Service, Department of Laboratory Medicine, National Institutes of Health Clinical Center, National Institutes of Health, Bethesda, Maryland, USA. IKAROS-associated diseases.

MAIN SYMPOSIUM 18: IMMUNE DISFUNCTION AND IMMUNOTHERAPY

Klaus Warnatz.
Center for Chronic Immunodeficiency. Center for Translational Cell Research, Department of Rheumatology and Clinical Immunology, Medical Center, University of Freiburg, Germany. With too little help of my friends. About pathogenesis of common variable immunodeficiency.
María Bellio.
Instituto de Microbiologia Paulo de Góes Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil. Cytotoxic CD4+ Tcells in Chagas disease. Friend of foe?
Naomi Taylor.
Pediatric Oncology Branch, National Institutes of Health, Bethesda, MD, USA. Integrating TCR-controlled fuzzy logic into CAR T cells to enhance therapeutic specificity.

MAIN SYMPOSIUM 19: SYSTEMS IMMUNOLOGY AND FUNCTIONAL GENOMICS

Rafael Argüello.
Aix Marseille Univ, CNRS, INSERM, CIML, Centre d'Immunologie de Marseille-Luminy, Marseille, France. New dimensions in single-cell biology: Metabolism meets epigenetics in epic-SCENITH.
Juan Fuxman Bass.
Biology Department, Boston University, Boston, MA, USA. Global landscape of human cytokine transcriptional regulation.
Otoniel Rodriguez Jorge.
Centro de Investigación en Dinámica Celular. Universidad Autónoma del Estado de Morelos, Mexico. Towards functional reprogramming of neonatal T cells: A systems immunology approach.

MAIN SYMPOSIUM 20: INFLAMMATION AND IMMUNE REGULATION

Guillaume Duménil.
Institut Pasteur, Université Paris Cité, INSERM UMR1225, Pathogenesis of Vascular Infections, Paris, France. Dermal Lyve1+FolR2+ perivascular macrophages act as sentinels against intravascular infections.
María Silvia Di Genaro.
Instituto Multidisciplinario de Investigaciones Biológicas de San Luis -CONICET-Universidad Nacional de San Luis. Argentina. Immunomodulation mechanisms in spondyloarthritis.
Paula Licona Limón.
Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, UNAM, Mexico. TIF1g regulates stability of Tregs during inflammation.

MAIN SYMPOSIUM 21: FOCIS SYMPOSIUM

Stephanie Eisenbarth.
Department of Medicine and Center for Human Immunobiology, Northwestern University Feinberg School of Medicine, Chicago, USA. Regulating the immune response to food allergens.
Susan Bueno.
Instituto Milenio de Inmunología e Inmunoterapia, Pontífica Universidad de Chile, Chile. Characterization of neutrophil subpopulations during a Streptpcoccus pneumoniae infection in mice.
Marcelo Hill.
Institut Pasteur de Montevideo, Uruguay and Immunobiology Department, Faculty of Medicine, Universidad de la República, Uruguay. TMEM176B as a druggable immunoregulator in cancer and autoimmunity.

MAIN SYMPOSIUM 22: AAIV SYMPOSIUM: ONE HEALTH

Margaret A. Liu.
ProTherImmune, USA; and Department of Medicine at Solna, Karolinska Institutet, Stockholm, Sweden. Gene-based vaccines and therapies for One Health.
Hugo Ortega.
Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral-CONICET, and Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Santa Fe, Argentina. Optimizing SARS-CoV-2 vaccine strategy: A One-Health perspective.
Elías Barquero Calvo.
Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica. Exploring innate immunity: Key to understanding chronic zoonotic infections.

MAIN SYMPOSIUM 23: INFECTION AND IMMUNITY

Jean Claude Sirard.
Universite de Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, U1019 - UMR9017 - CIIL - Center for Infection and Immunity of Lille, France. (France). Flagellin aerosol therapy as an immunomodulatory adjunct to the antibiotic treatment of drug-resistant bacterial pneumonia.
Nora A. Fierro.
Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico. Bilirubin, an unexpected ally in the immune response against hepatitis E virus.
Diana Hansen.
Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Australia. Integrated systems immunology approach identifies persistent blood monocyte dysfunction induced by both symptomatic and clinically silent Plasmodium vivax malaria.

MAIN SYMPOSIUM 24: CELLULAR AND MOLECULAR IMMUNOLOGY

Daniela Sauma.
Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Chile. Adenosine's impact on T cell exhaustion and the maintenance of cytotoxic responses in the tumor.
Gabriel Morón.
CIBICI-CONICET-Universidad Nacional de Córdoba, Argentina. The role of LSP1 in dendritic cells: Implications for antigen presentation, T cell activation, and tumor immunity.
Yvonne Rosenstein.
Instituto de Biotecnología, Campus Morelos, Universidad Nacional Autónoma de México, Mexico. Regulation of GLUT-1 expression and T-Cell activation by sialophorin (CD43).

MAIN SYMPOSIUM 25: HOST-PATHOGEN INTERACTION

Mauro Martins Teixeira.
Institute of Biological Sciences, Universidade Federal de Minas Gerais (UFMG) and Center for Advanced and Innovative Therapies, UFMG, Belo Horizonte, Brazil. Resolution of inflammation in the context of infection.
María del Pilar Aoki.
CIBICI-CONICET-Universidad Nacional de Córdoba, Argentina. Purinergic signaling subverts the immune response to Trypanosoma cruzi infection.
Laura Chiapello.
Facultad de Ciencias Químicas, Universidad Nacional de Córdoba (UNC) and Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET, Argentina. Cutaneous innate lymphoid populations drive IL-17A-mediated immunity in skin fungal infection.

MAIN SYMPOSIUM 26: MACROPHAGES SYMPOSIUM

Julie Helft.
Tumor associated macrophages and T cell immunity. Institut Cochin, Phagocytes and Cancer Immunology Laboratory, Université Paris Cité, INSERM U1016, CNRS, France.
Fernando Oneissi Martinez Estrada.
School of Biosciences and Medicine, University of Surrey, United Kingdom. Unifying considerations and evidence of macrophage activation mosaicism through human CSF1R and M1/M2 genes.

MAIN SYMPOSIUM 27: STEM CELL TRANSPLANTATION IN PATIENTS WITH INBORN ERRORS OF IMMUNITY

Pere Soler Palacín.
Pediatric Infectious Diseases and Immunodeficiencies Unit, Children's Hospital, Vall d'Hebron Barcelona, Spain. The importance of the assessment of immunological reconstitution after SCT in patients with IEI.

MAIN SYMPOSIUM 28: AUTOINFLAMMATORY DISORDERS

Cecilia Poli.
Faculty of Medicine, Clínica Alemana Universidad del Desarrollo, and Unit of Immunology and Rheumatology, Hospital Roberto del Río, Santiago, Chile. New autoinflammatory disorders.

MAIN SYMPOSIUM 29: LAMIG SYMPOSIUM 2

Eduardo Villablanca.
Division of Immunology and Allergy, Department of Medicine Solna, Karolinska Institute and University Hospital, Sweden. From Gut to Gums: Unraveling the link between intestinal inflammation and oral pathologies.
Alessandra Filardy.
Institute of Microbiology, Center for Health Science, Federal University of Rio de Janeiro, Brazil. Regulation of lung immune cell populations following acute exposure to cigarette smoke and pneumococcal infection by MerTk.
Julio Villena.

Laboratory of Immunobiotechnology. CERELA-CONICET, Argentina. Modulation of the innate immune memory in the respiratory mucosa by the local microbiota.

Araceli Pérez.

Laboratorio de inmunología e interacciones microorganismo-hospedero. UBIMED. Facultad de Estudios Superiores Iztacala, UNAM, Mexico. Role of the receptor CCR3 in lung inflammation during influenza A infection.

MAIN SYMPOSIUM 30: CANCER AND IMMUNITY

Florent Ginhoux.

Singapore Immunology Network Agency for Science, Technology, and Research, Singapore, Singapore. Myeloid cell heterogeneity in cancer.

Mario Ernesto Cruz Muñoz.

Facultad de Medicina, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico. Role and modes of action of SLAM family receptors in the anti-leukemia immune response.

Romina Gamberale.

Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Argentina. The role of T cells in Chronic Lymphocytic Leukemia (CLL).

MAIN SYMPOSIUM 31: AUTOIMMUNITY, AUTOINFLAMMATION AND IMMUNE REGULATION

Cecilia Poli.

Faculty of Medicine, Clínica Alemana Universidad del Desarrollo, and Unit of Immunology and Rheumatology, Hospital Roberto del Río, Santiago, Chile. Directed therapies in immune dysregulation.

Mehrnaz Mesdaghi.

Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Double negative T cells in ALPS and ALPS-like diseases.

Elena Hsieh.

Department of Pediatrics, Section of Allergy and Immunology, Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Children's Hospital Colorado, Aurora, Colorado, USA. Pediatric SLE: Clinical and immunological heterogeneity.

MAIN SYMPOSIUM 32: HOST-PATHOGEN INTERACTIONS 2

Marisa Gómez.

Centro de Estudios Biomédicos, Básicos, Aplicados y Desarrollo (CEBBAD), Departamento de Investigaciones Biomédicas y Biotecnológicas, Universidad Maimónides, Buenos Aires, Argentina. Understanding Staphylococcus aureus pathogenesis and the dual role of protein A induced inflammation.

Clive Gray.

Institute of Infectious Disease and Molecular Medicine, University of Cape Town; and Division of Immunology, Department of Biomedical Sciences, Biomedical Research Institute, Stellenbosch University, Cape Town, South Africa. How HIV impacts on placental and neonatal immunity.

Cristina Alonso-Vega.

Barcelona Institute for Global Health (ISGlobal), Bolivia. Congenital Chagas, more than just the simple passage of T. cruzi during pregnancy. Adaptations of the immune response in the mother and fetus.

MAIN SYMPOSIUM 33: IMMUNOTHERAPY AND VACCINES

Pierre Guermonprez.

Université de Paris, INSERM U1149, CNRS erl8252, Centre for Inflammation Research, Université de Paris Cité, Paris, France. Harnessing dendritic cell infiltration in solid tumors for cancer immunotherapy.

Faith Osier.

Department of Life Sciences, Imperial College London, London, United Kingdom. Leveraging human challenge studies for Malaria vaccine development.

Wendy Béguelin.

Division of Hematology & Medical Oncology, Weill Cornell Medicine, New York, USA. Improving T-cell immunotherapies against B-cell lymphomas by inhibiting EZH2.

MAIN SYMPOSIUM 34: IMMUNE DEFENSE MECHANISMS

Enzo Poirier.
Innate Immunity in Physiology and Cancer laboratory, Institut Curie, PSL Research University, Paris. France. Uncovering the ancestral immune modules shared across domains of life.
Jose Carlos Alves-Filho.
Department of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Brazil. Control of regulatory T cell differentiation and stability during inflammation.
Dario Zamboni.
Department of Cellular and Molecular Biology, Ribeirão Preto Medical School, University of Sao Paulo, Brazil. Inflammasome activation in response to intracellular pathogens and its implications for disease development and infection control.

MAIN SYMPOSIUM 35: CLINICAL IMMUNOLOGY AND IMMUNOTHERAPY

Silvia Sanchez Ramón.
Departamento de Inmunología Clínica, Instituto de Medicina de Laboratorio, Hospital Clínico San Carlos, Universidad Complutense of Madrid, Spain. Immunological assessment in fertility disorders.
Belinda Sánchez Ramirez.
Immunology and Immunotherapy Direction, Center of Molecular Immunology, Havana, Cuba. Cancer Immunotherapy: 30 years of experiences from the Center for Molecular Immunology
Kenneth Gollob.
Translational Immuno-oncology Laboratory, Center for Research in Immuno-oncology – CRIO, Hospital Israelita Albert Einstein, São Paulo, SP Brazil. Identifying Biomarkers and Mechanisms of Immunotherapy Response in Metastatic NSCLC.

MAIN SYMPOSIUM 36: ALLERGY

Winfried Pickl.
Division of Cellular Immunology and Immunohematology at the Institute of Immunology, Medical University of Vienna, Austria. On the modulation of allergen-specific T cell responses: the importance of interleukin-2.
Joana Neves.
Centre for Host-Microbiome Interactions, King's College London, United Kingdom. Modulation of the intestinal cellular environment by innate lymphoid cells.
Musa Khaitov.
NRC Institute of Immunology FMBA Russia, Moscow, Russioan Federation. Recombinant vaccine against birch pollen allergy.

MAIN SYMPOSIUM 37: TRANSLATIONAL IMMUNOLOGY

Autoimmunity & Immunotherapy, FOCIS Center of Excellence PAn'THER, Rouen, France. IFNγ causes mitochondrial dysfunction and oxidative stress in myositis.
Tim Sparwasser.
Institute of Medical Microbiology and Hygiene, University Medical Center of Johannes Gutenberg University, Mainz, Germany. Metabolic checkpoints for the treatment of autoimmune disease.

MAIN SYMPOSIUM 38: IMMUNODEFICIENCIES II

Klaus Warnatz.
Division of Immunodeficiency, Department of Rheumatology and Clinical Immunology and Center for Chronic Immunodeficiency, Medical Center, University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany. Anything targeted of something common and variable? Treatment concepts for noninfectious complications in CVID.

OPENING LECTURES IN SELECTED ABSTRACT PRESENTATIONS 1

María de los Ángeles Serradell. Cátedra de Microbiología, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina. Effect of a kefir-isolated *Lentilactobacillus kefiri* strain in different models of inflammation-associated disorders.

SELECTED ABSTRACTS PRESENTATIONS SESSION 1

Alonso Lira. *(Universidad de Chile, Chile. Role of IRE1 RNase activity in the degradation of miRNAs in conventional type 1 dendritic cells.*

Hai Huang. *Feinstein Institutes for Medical Research, USA. Exercise Therapy Suppresses MASH Development by Modulating Hepatic Regulatory T-cells.*

Guadalupe Suarez. *INSERM, PSL University, Institut Curie, France. Epigenetic control of CD8+ T cell tissue homing and tissue resident memory T cell precursors differentiation by the histone methyltransferase SUV39H1.*

Luis Carlos Ruelas Ruiz. *Departament of Immunology, Faculty of Medicine, Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, México. Diethylcarbamazine regulates cytokine production and reduces the viral titer in epithelial cells infected by the influenza A (H1N1)pdm09 virus.*

OPENING LECTURES IN SELECTED ABSTRACT PRESENTATIONS 2

Nadia Bannoud. IHEM-CONICET, Facultad de Ciencias Médicas UNCUYO, Argentina. *Circulating galectin-1 in melanoma patients under antiangiogenic treatment: clinical implications.*

SELECTED ABSTRACTS PRESENTATIONS SESSION 2

Juliana Lima de Souza. *Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, IBqM/UFRJ, RJ, Brazil. Neutrophil Extracellular Traps (NETs) drive a chemoresistant phenotype in human breast cancer cells through PI3K / AKT / NF-kappaB pathway.*

Valeria da Costa. *The Kennedy Institute of Rheumatology, Nuffield Department of Orthopedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom. Cytoplasmic protein associates to PD-L1 in cell surface checkpoint domains assembling a novel target for antibody response in long term survivors of pancreatic cancer.*

Maynara Santana-Gonçalves. *University of São Paulo, Ribeirão Preto, Brazil. Immune reconstitution after autologous stem cell transplantation is associated with clinical outcomes in patients with systemic sclerosis.*

Ángeles Romina Arena. *Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires. Ketamine reduces peripheral and brain inflammation by promoting regulatory immune cells, alleviating LPS-induced depressive-like behavior in mice.*

OPENING LECTURES IN SELECTED ABSTRACT PRESENTATIONS 3

Paula Soledad Pérez. *Instituto de Investigaciones Biomédicas en Retrovirus y Sida (INBIRS, UBA-CONICET), CABA, Argentina. Regulation of inflammation by plasma extracellular vesicles.*

SELECTED ABSTRACTS PRESENTATIONS SESSION 3

Joseana de Oliveira. *Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Brazil. HIF2-alpha Activity in Myeloid Leukocytes Promotes Susceptibility to Mycobacterium tuberculosis Infection.*

Maycon Tavares Emílio-Silva. *Department of Structural and Functional Biology, Institute of Bioscience, São Paulo State University, Botucatu, São Paulo, Brazil. CITRAL, a monoterpene that protects against the intestinal and systemic damage caused by high-fat diet and lipopolysaccharide in mice.*

Fernando Nicolás Sosa. *IMEX -CONICET-Academia Nacional de Medicina, Argentina. IL-10 Modulates Susceptibility to Shiga Toxin Type 2 and Leukocyte Dynamics in IL-10-Deficient Mice.*

Daniela Olivera. *Laboratory of Immunoregulation and Inflammation, Institut Pasteur de Montevideo / Immunobiology Department, Faculty of Medicine, University of the Republic, Montevideo, Uruguay. The cation channel TMEM176B is a druggable protective factor in autoimmune disease.*

OPENING LECTURES IN SELECTED ABSTRACT PRESENTATIONS 4

Angel Justiz Vaillant (West Indies). *Systematic review about the diagnosis of transient hypogammaglobulinemia of infancy.*

SELECTED ABSTRACTS PRESENTATIONS SESSION 4

Shokrollah Elahi. *University of Alberta, Canada. Polyfunctional CD8+CD226+RUNX2hi effector T cells are diminished in advanced stages of chronic lymphocytic leukemia.*

Vera Tifner. *Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires, Argentina. Immune characterization of Major Depressive Episode: imbalanced Th1/Th17/ CD4+IL-10+ T cells and increased activation and exhaustion of CD8+ lymphocytes.*

Gloria Soldevila. *Instituto de Investigaciones Biomédicas. Universidad Nacional Autónoma de México, Mexico. Vitamin C promotes a stable phenotype and demethylated TSDR Foxp3 of in vitro expanded allospecific iTregs.*

Yago A. Arribas. *Institut Curie, PSL University, Inserm U932, Immunity and Cancer, France. Non-canonical proteome derived from transposable elements is a source of functional protein isoforms and cancer neoantigens.*

OPENING LECTURES IN SELECTED ABSTRACT PRESENTATIONS 5

Gerardo Pavel Espino Solís. *National Laboratory of Flow Cytometry, Faculty of Medicine, and Biomedical Sciences, Universidad Autónoma de Mexico, Mexico. Immune monitoring of pediatric patients with tick-borne diseases.*

SELECTED ABSTRACTS PRESENTATIONS SESSION 5

Lena Peter. *Berlin Institute of Health Center for Regenerative Therapies, Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Berlin, Germany. Impact of CRISPR-Cas9-mediated FoxP3 gene knockout on the potency of therapeutic effector T-cells.*

Alejandra Chaparro. *Department of Oral Pathology and Conservative Dentistry, Periodontics, Faculty of Dentistry. Universidad de Los Andes. Santiago, Chile. The systemic spread of periodontal bacteria outer membrane vesicles disrupts glucose tolerance, induces systemic inflammation and adverse pregnancy outcomes*

Federico Páez Córdoba. *Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín-CONICET, Argentina. Booster vaccination with a recombinant subunit vaccine against SARS-CoV-2 increases antigen specific mucosal antibodies.*

Filipe Pereira-Dutra. *Fundação Oswaldo Cruz, Brazil. Sepsis-induced lipid droplets contributed to liver disfunction and to resistance to bacterial infection.*

OPENING LECTURE IN SELECTED ABSTRACT PRESENTATION SESSION 6.

Maite Duhalde Vega. *IQUIFIB-CONICET, Universidad de Buenos Aires, Argentina. Tryptophan metabolism: A key player in host-microbiome interactions and diet-induced inflammation.*

SELECTED ABSTRACTS PRESENTATIONS 6.

Diego Delgado Zaldívar. *Departamento de Inmunología y Reumatología, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, UNAM, Mexico. Role of TGF-Beta 3 and TCR-gamma delta T cells in oral tolerance.*

Julio Santelices. *Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Chile. Identifying immune cell subpopulations in hantavirus cardiopulmonary syndrome through bulk RNA-seq deconvolution.*

Pedro Paulo Diniz Lucinda. *Federal University of Minas Gerais, Brazil. Single-cell RNA sequencing and multiparameter flow cytometry data reveal highly cytotoxic and inflammatory profile from gamma-delta T cells from Chagas disease cardiomyopathy patients.*

Fengyi Wan. *Johns Hopkins University, USA. Complement in breast milk promotes infant health via shaping offspring's evolving gut microbiota.*

OPENING LECTURE IN SELECTED ABSTRACT PRESENTATION SESSION 7.

Estefania Nova. *Departamento de Química, Facultad de Ciencias Naturales, Matemáticas y Medio Ambiente, Universidad Tecnológica Metropolitana, Santiago, Chile. Mitochondrial transfer, a potential mechanism of CD4+ T cell exhaustion in oral cancer.*

SELECTED ABSTRACTS PRESENTATIONS 7.
Jesús Daniel Zambrano. *Institute of Cellular Physiology, UNAM, México. TGF-beta modulates the TCR signal strength in CD8+ T cells in a TIF1gamma -dependent manner.*
Julia Castro. *CT-Vacinas, Brazil. ASP-2/Trans-sialidase chimeric protein induces robust protective immunity in experimental models of Chagas disease.*
Surojit Sarkar. *University of Washington School of Medicine, USA. Engineering CAR-T cells for durable control and checkpoint blockade synergy in solid tumors.*
Rodrigo Tonalli Camacho-Pacheco. *National Perinatology Institute, Mexico. Characterization of the adaptative immune response in HIV exposed uninfected infants.*

AINCA SELECTED ABSTRACTS ORAL PRESENTATIONS

Celina Andrea Franco. *Hospital Prof. Dr. J. P. Garrahan, CABA, Argentina. IKZF1 Dominant Negative and uncommon immune-dysregulation: Case report.*
Lucia Peirano. *Centro de Inmunologia Bezrodnik. Hospital Italiano de Buenos Aires. Hospital El Cruce, Pcia. Buenos Aires, Argentina. ADA 2: A symptomatic heterozygous patient. Case report.*
María Pilar Tejada. *Hospital General de Niños Ricardo Gutiérrez, CABA, Argentina. What we have lernt about Hematopoietic Cell Transplantation in Wiskott-Aldrich Syndrome: clinical features and immune reconstitution.*

VETERINARY IMMUNOLOGY ROUND TABLE

José Angel Gutierrez Pabello. *Departamento de Microbiologia e inmunologia, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico. Cell death in bovine tuberculosis: bacterial dissemination or control?*
Juan José Duarte. *Facultad de Ciencias Veterinarias, UBA, Argentina. Preliminary evaluation of vaccines designed against Mycobacterium avium subsp. Paratuberculosis: immune response and protection in challenged mice.*
Florencia Belén Lobo Gaitán. *Instituto de Agrobiotecnología y Biología Molecular (IABIMO), Argentina. New-generation vaccines against foot-and-mouth disease virus: strategies to enhance the immunogenicity of recombinant empty capsids.*

AINCA CLINICAL CASES DISCUSSIONS 1.

Gabriela Pereyra. Hospital General *San Martin de La Plata, Buenos Aires, Argentina.*
María Gabriela Vazquez Ortuño. *Hospital General de Agudos "Carlos G.Durand"-CABA, Argentina.*

AINCA CLINICAL CASES DISCUSSIONS 2: IEI TREATMENT (BIOLOGICS TREATMENT, HSCT).

Matías García. *Centro de Inmunologia Clínica Dra.Liliana Bezrodnik y Equipo-CABA, Argentina.*
Miguel Galicchio. *Hospitla de Niños "Victor J.Vilela" de Rosario, Santa Fe, Argentina.*
María Pilar Tejada. *Hospital de Niños "Dr.Ricardo Gutierrez"-CABA, Argentina.*

SELECTED ABSTRACTS FOR PRIZES

BEST WORK IN BASIC IMMUNOLOGY (BWBI) PRIZE

Ignacio Cebrián. *Instituto de Histología y Embriología de Mendoza (IHEM) - Universidad Nacional de Cuyo - CONICET, Mendoza, Argentina. Dendritic cell phagosomes recruit GRASP55 for optimal transport of antigen-loaded MHC molecules to the plasma membrane.*

Gerardo Suarez-Rojas. *Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán/Department of Immunology and Rheumatology, Mexico. Helios limits CD8 T cell antitumor capacity.*

Karen Álvarez Díaz. *Grupo de Inmunología celular e Inmunogenética- Universidad de Antioquia, Colombia. Targeted Inhibition of Pro-Inflammatory Slan+ Monocytes Using WGA-Functionalized Nanoparticles Encapsulating Itacitinib: A Novel Therapeutic Approach.*

Carolina Abrate. *Departamento de Bioquímica Clínica, CIBICI-CONICET-Universidad Nacional de Córdoba, Argentina. CD39: A Key Regulator of Tumor Progression and a Promising Therapeutic Target.*

BEST WORK IN CLINICAL AND TRANSLATIONAL IMMUNOLOGY (BWCTI) PRIZE

Macarena Lépez. *Pontificia Universidad Católica de Chile, Chile. Association between Maternal Obesity and the Phenotype of Monocytes and Hematopoietic Progenitor Cells in their Offspring at Birth.*

Gabriela Yamazaki de Campos. Department of Cell and Molecular Biology and Pathogenic Bioagents, Ribeirão Preto Medical School, University of São Paulo, Brazil. Bioengineering mannan-specific CAR-NK cells for enhanced antifungal immunity against invasive candidiasis.

Lorenzo Erra. Departamento de Fisiología, Biología Molecular y Celular, Instituto de Biociencias, Biotecnología y Biología Traslacional (IB3) e Instituto de Química Biológica (IQUIBICEN), FCEN, UBA, CONICET, CABA, Argentina. Whole Exome Sequencing in Argentine Patients with Inborn Errors of Immunity: A Comprehensive Study.

Daiana Soledad Flores. Instituto de Química Biológica (IQUIBICEN)-FCEN-UBA-CONICET, CABA, Argentina. Functional Assessment of Novel STAT3 Genetic Defects in Argentinean Patients with Inborn Errors of Immunity.

LEONARDO SATZ PRIZE (SAI)

Gonzalo Cabrerizo. *Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS) - Universidad de Buenos Aires - CONICET, CABA, Argentina. Prostaglandin E2 drives a unique pro-resolving profile in human macrophages.*

María Sol Martinez. *FOCIS Center of Excellence Centro de Inmunología Clínica de Córdoba (CICC). CIBICI-CONICET. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba, Argentina. Semen inflammation to fertility challenges: Unraveling the immune cross-talk between male and female genital tracts and its impact on offspring.*

Jeremias Dutto. *CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. Understanding the immune dysregulation in children with Down Syndrome: are CXCR3+ T follicular helper cells involved in the autoimmune-prone profile reported in these population?*

Alexia Vereertbrugghen. *Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina, Buenos Aires, Argentina. CD4+ T cells drive corneal nerve damage but are dispensable for corneal epitheliopathy development in the context of dry eye.*

ADAPTIVE IMMUNITY

1. 003. **MODULATION OF IFN-γ AND IL-17 PRODUCTION IN THYMIC AND PERIPHERAL γδT CELLS BY IGG FROM ATOPIC DERMATITIS PATIENTS: IMPLICATIONS FOR HOMING AND EPIGENETIC MECHANISMS**

Nicolle Rakanidis Machado[1], Beatriz Fagundes Oliveira[1], Valéria Aoki[1], Raquel Leão Orfali[1], Jefferson Russo Victor[1,2]

1. Laboratory of Medical Investigation LIM-56, Division of Dermatology, Medical School, University of São Paulo, São Paulo 05403-000, Brazil

2. Post Graduate Program in Health Sciences, Santo Amaro University (UNISA), São Paulo 04829-300, Brazil

Atopic Dermatitis (AD) is a multifactorial skin disease characterized by barrier dysfunction, immune response alterations, and polarization toward a Th2 pattern. gamma-delta T cells (γδT cells) are significant producers of IFN-γ, IL-17, and IL-22, potentially influencing AD development. This study aimed to assess the impact of IgG from AD patients on human peripheral γδT cells in vitro. Thymocytes from 0-3-day-old infants and peripheral blood mononuclear cells (PBMCs) from adults were cultured with 100 µg/mL of IgG purified from moderate-severe adult AD patients. As controls, we employed a mock condition, IgG intended for therapeutic use, or IgG purified from healthy individuals. All IgG formulations exhibited comparable levels of IgG subclasses (IgG1-IgG4) and demonstrated interaction with thymic γδT cell membranes. IgG from AD donors elicited increased IFN-γ, IL-17, IL-22, and cutaneous lymphocyte antigen (CLA) expression while reducing α4β7 expression on thymic γδT cells. Conversely, only IFN-γ and IL-17 effects were observed in peripheral γδT cells in response to AD IgG. Furthermore, we evaluated miRNA expression in cultured thymocytes via Next-Generation Sequencing (NGS) and identified nine differentially expressed miRNAs in response to IgG from AD donors. These findings suggest that IgG from AD donors can modulate infant γδT cell maturation and adult γδT cell function, potentially contributing to AD development through mechanisms involving epigenetic regulation mediated by miRNAs.

2. 024. **GLUCOCORTICOIDS REDIRECT NAÏVE T CELLS TO THE BONE MARROW FOR PRESERVATION IN MALNOURISHED MICE**

Melanie Gubbels Bupp[1], Jacob Hanes, Clay Phillips[1], Takesha Foster[1], Kwesi Dadzie[1]

1. Randolph-Macon College

Malnutrition is associated with reductions in the number and function of peripheral T cells. It is unknown whether malnourishment results in the increased apoptosis of naïve T cells or whether peripheral naïve T cells are directed to another lymphoid organ such as the bone marrow, which has been shown to be a protective environment in other contexts. Therefore, we evaluated the number of naïve T cells in the bone marrow and lymph nodes of malnourished and control mice. We found that malnourished mice demonstrated enhanced naïve T cell residency in the bone marrow and diminished T cell migration to the lymph nodes. Adoptive transfer studies revealed that malnutrition has intrinsic effects on naïve T cells; control naïve T cells entered the lymph nodes and spleen more efficiently than malnourished T cells regardless of the malnutrition status of the recipient, while malnourished naïve T cells entered the bone marrow more efficiently and experienced half as much cell death there as compared to control T cells, regardless of recipient. Experiments with mice conditionally deficient for the glucocorticoid receptor in T cells demonstrated that T cell sensitivity to glucocorticoids is required for malnutrition-induced T cell migration to the bone marrow. Moreover, malnourished naive CD4+ T cells overexpress CXCR4 and CCR7 in the spleen and bone marrow, respectively, as compared to well-nourished T cells. However, malnourished naive CD4+ T cells lacking the glucocorticoid receptor express similar levels of both chemokine receptors as control T cells. Overall, we have determined that excess glucocorticoids secreted during malnutrition redirect naïve T cells to the bone marrow where they experience less cell death, perhaps due to differences in chemo-

kine receptor expression, which may establish a modified migration pattern that allows for naïve T cell preservation in the bone marrow as well as partial preservation of immunosurveillance.

3. 034. **TRANSCRIPTOMIC PROFILE OF NEONATAL CD4+ T CELL AS A FUNCTION OF GESTATIONAL AGE AND TYPE OF BIRTH**

Linda Aimara Kempis Calanis[1], Carlos Jesus Ventura-Martínez[1], Otoniel Rodríguez Jorge[1], Salvatore Spicuglia[2]

1. Centro de Investigación en Dinámica Celular, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, 62210 Cuernavaca, México.

2. Aix-Marseille University, Inserm, TAGC, UMR1090, 13288 Marseille, France

Prematurity is the leading cause of neonatal death worldwide. Most premature newborns are born by cesarean section. Cesarean delivery is a life-saving procedure that reduces the risk of maternal and infant morbidity and mortality when medically indicated. The World Health Organization (WHO) recommends that this method not exceed 15% of births in the country. According to preliminary data from Secretaria de Salud (SINAC, in 2020) in Mexico, the number of births by cesarean section exceeded natural births reaching more than 50%. We wanted to know if the type of birth or gestational age (premature birth) influences the establishment of the neonatal gene expression profile. To characterize the immune response of neonatal T lymphocytes, we performed a transcriptomic (RNA-seq) analysis of naïve CD4+ T cells from neonates. We found that, in the birth transition, the type of delivery determines the establishment of the gene expression profile that the newborn will have, since the CD4+ T lymphocytes of neonates born by cesarean delivery at term present a different gene expression profile than those of neonates born by natural birth, by 600 differentially expressed genes. The overexpressed genes in neonatal CD4+ T cells born by natural birth were enriched in pathways of Immune response (cytokine-cytokine receptor interaction, Th17 pathway), activation of several signaling pathways (NF-kB, TNF, NOD, MAPK) and those of several diseases. This could mean that natural birth may induce the activation response of CD4+ T cells. On the other hand, premature neonates do not change their gene expression pattern significantly as compared to full-term neonates, born in both cases by cesarean section. Our findings indicate that for CD4+ T lymphocytes, the type of birth is extremely important in establishing the gene expression profile of the neonate, having a greater impact than the last weeks of gestation.

4. 061 **TGF-BETA MODULATES THE TCR SIGNAL STRENGTH IN CD8+ T CELLS IN A TIF1GAMMA -DEPENDENT MANNER**

Jesús Daniel Zambrano[1], Eugenio Contreras[1], José Luis Ramos, Paula[1]

1. Institute of Cellular Physiology, National Autonomous University of Mexico

TGF-β regulates the quiescence and activation of naïve CD8+ T cells upon antigen exposure. The E3 ubiquitin ligase TIF1γ controls a Smad4-independent arm of the TGF-β signaling pathway. However, the role of TIF1γ in CD8+ T cells remains unknown. Here, we deleted TIF1γ from CD8+ T cells in mice to elucidate its function in the context of TGF-β. In vitro, TGF-β prevented the proliferation and survival of activated WT CD8+ T cells but not of their TIF1γ-/- counterparts, which correlated with higher c-MYC and BCL-2 expression in the knockouts. Similarly, TGF-β inhibited Irf4 and Nur77 expression in WT cells activated with varying degrees of TCR stimulation, but not in TIF1γ-/- cells. Western blot analyses of TCR signaling components revealed increased p-ERK and p-p38 in TGF-β-stimulated CD8+ TIF1γ-/- cells upon TCR activation compared to WT, suggesting a critical role of TIF1γ in regulating the TCR signal strength in CD8+ T cells. In vivo, naïve CD8 KO cells transferred into RAG2-/- mice displayed an effector phenotype with increased proliferation and activation, compared to WT cells. Strikingly, CD8+ T cells from Tif1γfx/fx CD8 Cre mice infected with OVA-expressing Listeria monocytogenes proliferated more and had higher numbers of KLRG1+ CD8+ T cells in blood during the clonal expansion phase, compared to CD8+ T cells from WT mice. Additionally, we found higher numbers of effector memory T cells (Tem) in the blood, spleen, and liver of TIF1γ-deficient mice 30 days after a second exposition with the pathogen. This suggests that TIF1γ regulates CD8+ T cell activation and differentiation into Tem. Together, this data demonstrates a novel function of TIF1γ: by modulating the TCR signaling, it regulates CD8+ T cell activation. Therefore, TIF1γ might be a novel target to enhance the immune response to vaccines against viruses and bacteria.

5. 062 **CHARACTERIZATION OF THE RE-**

PERTOIRE OF SERUM IMMUNOGLOBULINS IN INFECTED INDIVIDUALS

Vicente Bozza[1], Danielle Rodrigues[1], Barbara Gabrielle[1], Andre Vale[1]
1. Universidade Federal do Rio de Janeiro

The humoral immune response depends on the activation of B cells that can diferentiate into antibody-producing plasma cells. The set of B cells and secreted immunoglobulins composse a very diverse repertoire, which is derived from genetic factors and selection mechanisms during their development and influenced by enviromental factors. The study of the immunoglobulin repertoire is very pertinent since infections and therapeutic interventions can cause disturbances in the immunoglobulin repertoire. The present work aims to analyse and map the serum immunoglobulin repertoire in individuals infected with SARS-CoV-2, allowing the establishment of possible correlations between signatures in the humoral response with different clinical outcomes of COVID-19. One of the techniques used in this work was the modified immunoblot, which consists of evaluating the immunorreactivity of serum samples from infected individuals against a panel of unrelated antigens, such as E. Coli and Calu cell extracts. In parllel, we intend to investigate whether the therapeutic strategy of plasmapheresis adopted in severe cases interferes with the global repertoire of immunoglobulins. It was possible to observe differences in the immunoreactivity profile of serum IgG agains E. Coli antigens when we compared groups of infected and uninfected individuals, suggesting an alteration in the clonal repertoire due to infection. We also observed differences in the immunoreactivity profile among infected individuals stratified according to the severity of the desease, with greater intensity in serum IgG immunoreactivity agains unrelated antigens in individuals with severe COVID-19 in relation to individuals with mild symptoms. Disturbances in the serum IgG immunoreactivity repertoire has been observed in patients after transfusion of convalescent plasma, suggesting that donor plasma is capable of inducing plasma cell differentiation in recipient individual. These data bring light to further studies on the importance of the immunoglobulin repertoire for the development of vacines and treatments for COVID-19 and other infectious deseases.

6. 091 **DENDRITIC CELL PHAGOSOMES RECRUIT GRASP55 FOR OPTIMAL TRANSPORT OF ANTIGEN-LOADED MHC MOLECULES TO THE PLASMA MEMBRANE**

Ignacio Cebrián[1], Sofía Dinamarca[1], María Jesús Pena Rodríguez[2], Elena Priego[3], Nathalie Brouwers[2], Martina Barends, Jamina Brunnberg[4], Robert Tampé[4], Nicolas Blanchard5, David Sancho[3], Vivek Malhotra[2]
1. IHEM-CONICET, Argentina
2. Centre for Genomic Regulation (CRG), Spain
3. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Spain
4. Institute of Biochemistry, Biocenter, Goethe University Frankfurt, Germany
5. Toulouse Institute for Infectious and Inflammatory Diseases (Infinity), France

Background: Dendritic cells (DCs) are highly adapted to present exogenous antigen-derived peptides in association with MHC class I and II molecules, which triggers activation of CD8+ and CD4+ T lymphocytes, respectively. The former process is called cross-presentation, while the latter is the classical MHC-II presentation pathway. The endocytic network is essential for antigen processing to generate peptides that are loaded onto MHC molecules. However, the final steps in the traffic of MHC/peptide complexes from the endocytic system to cell surface remain poorly understood. Objective: The main objective of this study was to explore the role of the Golgi reassembly-stacking protein of 55 kDa (GRASP55) during the phagosomal export of antigen-loaded MHC molecules. This research's rationale is that GRASP55 regulates unconventional protein secretion, and this pathway shares similarities with the exogenous antigen presentation pathways. Methods: We performed different flow cytometry-based experiments, antigen presentation assays, confocal microscopy analysis, and biochemistry techniques to address this study in wild type (WT) and GRASP55-/- bone marrow-derived DCs (BMDCs). Results: We show that GRASP55 plays an essential role in antigen presentation. Using a model antigen coated to latex beads, soluble or associated to Escherichia coli, we found that both MHC-I cross-presentation and MHC-II antigen presentation are significantly inhibited in BMDCs of GRASP55-/- C57BL/6 mice compared to WT DCs. GRASP55 was recruited to late DC phagosomes and necessary for efficient sorting of loaded MHC-I and -II molecules from phagosomes to the plasma membrane.
Conclusion: Our data show that GRASP55 is crucial for the intracellular transport of MHC-I and -II molecules in the process of exogenous antigen

presentation.

7. 120. **INTERLEUKIN 9 EXPRESSION IS REGULATED BY TRIM33 IN TH9 AND REGULATORY T LYMPHOCYTES**

Javier Orozco-Córdoba[1], Eugenio Contreras-Castillo[1], José Luis Ramos-Balderas[1], Paula Licona-Limón[1]

1. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico

The TGF-β signaling pathway has been extensively studied in CD4+ T cell subsets, including Th17, Tfh and Treg, however, its role in regulating Th9 biology and IL-9 secretion remains unclear. Trim33 is a E3-ubiquitin ligase, as well as a double epigenetic reader protein (PHD and Bromo domains) that interacts with Smad2/3 and thus, regulates a non-canonical pathway of TGF-β that has remained mainly unexplored in immune populations. Recently, it has been described that other T cell subsets besides Th9 are able to secrete IL-9, such as, Tregs, but this is still controversial. Thus, we aimed to dilucidated the role of Trim33 during the differentiation and function of Th9 cells and its role on the production of IL-9 on Th9 and Treg cells, by using *in vitro* and *in vivo* models using a conditional knockout mice model for Trim33 (CD4-Cre) on the IL-9, IL-4 and FOXP3 reporter background. Our data demonstrates that Trim33 plays a crucial role in the differentiation and function of Th9 cells by inhibiting IL-9 secretion and Th9 differentiation, suggesting an early role for Trim33 in Th9 linage commitment. In an *in vivo Nippostrongylus brasiliensis* infection model, Trim33 deficiency enhances a more efficient type 2 immune response and increase the generation of Th9 and Th9-like Tregs, a newly and poorly described population characterized by the concomitant expression of IL-9 and FOXP3, additionally, in a B16-OVA tumor model, both Th9 and Th9-like Treg cells were observed. These findings might suggest that Trim33 regulates multiple signaling pathways beyond TGF-β by modifying IL-9 production and thus, suppressing the generation of Th9 and Th9- like Treg cells, characterized as an intermediate population between conventional Th9 and Treg cells. Further studies are needed to fully characterize these cell populations and to elucidate Trim33-dependent regulatory mechanisms in these cells.

8. 223. **IMPAIRED ADAPTIVE IMMUNE RESPONSE IN THE UPPER RESPIRATORY TRACT DUE TO PROTEIN DEFICIENCY: BENEFITS OF NASAL ADMINISTRATION OF IMMUNOBIOTICS**

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Due to the immune cell composition and function in nasopharynx-associated lymphoid tissue (NALT), the development of efficient nasally administered immunotherapies could be promoted. The nasal priming of malnourished mice with the peptidoglycan (PG) of *Lacticaseibacillus rhamnosus* CRL1505 (Lr) is as effective as a viable strain for improving immune response against *Streptococcus pneumoniae* (Sp). However, the impact of these treatments on NALT is unknown. Here, we compare antigen-presenting cells (APCs) and T and B cell subsets in the NALT during the specific immune response in malnourished mice treated nasally with Lr or PG. Weaned Swiss mice were malnourished with a protein- free diet (PFD) for 21d. Then, malnourished mice received a balanced conventional diet (BCD) during 7d or BCD with nasal supplementation with Lr or PG during the last 2d of treatment. Malnourished control mice received PFD. Well-nourished control group consumed BCD. On d8, all groups were infected with Sp (10^7 cells/mouse). APCs of NALT were studied on d2 post-infection. T and B cell populations of NALT and the production of specific antibodies were examined on d10, d14, and d21 post-infection. After infection, malnourished mice showed a significant reduction in the number of total cells, T and B lymphocytes, and the APCs in NALT. Unlike BDC, Lr was able to normalize the number of $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ lymphocytes. Furthermore, Lr and PG treatments induced an increase in total B cells ($B220^+$cells), mature B cells ($B220^{high}CD4^{Low}IgM^+$), and immature B cells ($B220^{Low}CD4^{High}IgM^-$). Moreover, mice receiving Lr or PG showed higher levels of anti-pneumococcal IgA and IgG in nasal lavage than the other mice, especially on d21. Malnourished animals did not show Igs switching in the period studied. These results highlight the importance of NALT as a target for the administration of postbiotics and immunobiotics to enhance respiratory immunity in immunocompromised malnourished hosts.

9. 234. **IMMUNE CHARACTERIZATION OF MAJOR DEPRESSIVE EPISODE: IMBALANCED TH1/TH17/ CD4+IL-10+ T CELLS AND INCREASED ACTIVATION AND EXHAUSTION OF CD8+ LYMPHOCYTES**

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Depression is a highly prevalent psychiatric condition referred to as a Major Depressive Episode (MDE). Its underlying causes and mechanisms are not well understood, leading to limited effectiveness of current treatments. Inflammation has been suggested to play a role in MDE pathophysiology through altered immune responses. This study aimed to investigate the responses of effector CD4+ T helper (Th) cell subsets and the activation and exhaustion status of CD8+ lymphocytes in the peripheral blood of MDE patients. 57 patients were evaluated by psychiatrists using the International Psychiatry Interview MINI to diagnose MDE and the Hamilton Depression Rating Scale (HADRS) to define the episode's severity. The MDE sample was 26% male and 74% female with 18 to 55-year age range and matched with the HC group (N=34) by age and gender. Blood samples (10 mL) were collected for peripheral blood mononuclear cells (PBMC) isolation, and direct staining. The intracellular production of IFNγ (Th1), IL-17 (Th17), and IL-10 ($CD4^+IL\text{-}10^+$ T cells) was analyzed in stimulated PBMCs, and the activation status of CD8+ lymphocytes was evaluated in 100 µL of blood by direct immunophenotyping, and analyzed by flow cytometry. No significant differences were found in the frequencies of Th1 and Th17 cells, but MDE patients showed a significantly lower percentage of CD4+IL-10+ T cells ($p<0.05$) and CD4+IFNγ+IL-10+ cells ($p<0.01$) compared to HC. The reduction in the CD4+IL-10+ T cells and CD4+INFγ+IL-10+ population negatively correlated with the number of depressive episodes ($p<0.05$ and $p<0.01$, respectively) and duration of illness ($p<0.01$). Additionally, a higher proportion of activated CD8+CD69+ cells ($p<0.0001$) with an exhausted phenotype (CD8+PD1+LAG3+) ($p<0.0001$) was observed, which positively correlated with severity, number of episodes, and illness duration in MDE compared to HC. In conclusion, MDE patients exhibit an imbalance in Th1/Th17/CD4+IL-10+ T cells and increased activation and exhaustion of cytotoxic CD8+ lymphocytes.

10. 239. **THE ROLE OF CLUSTERIN IN DENDRITIC CELL FUNCTION**

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Clusterin is a multifunctional glycoprotein involved in physiological and pathological processes such as apoptosis, protein homeostasis, Alzheimer's disease, and cancer. Previous research demonstrated that clusterin protects mature dendritic cells (DCs) from reactive oxygen species (ROS)-induced cell death. Here, we investigate the broader mechanisms by which clusterin influences DC survival and function. We examined whether clusterin protects DCs from additional pro-apoptotic stimuli. Monocyte- derived DCs, either expressing clusterin (LPS-treated, 10 ng/ml) or not (controls), were subjected to stress conditions: heat shock (42°C, 5-20 min, followed by 24 h at 37°C), ER stress (brefeldin A, 0.1-10 mM, 12 h), hypoxia ($CoCl_2$, 0.02-2 mM, 24 h), UV radiation (exposure for 30 s to 2 min, followed by 24 h culture), and oxidative stress (tert-Butyl peroxide, 25-100 µM, 24 h). DC viability was assessed by Annexin V and propidium iodide staining via flow cytometry. Results showed that mature DCs had significantly higher resistance to oxidative stress compared to immature DCs ($n=3\text{-}5$, $p<0.01$). Conversely, mature DCs exhibited lower resistance to

hyperthermia and UV light (n=3-5, p<0.01). To explore clusterin's role in DC function, we performed co-immunoprecipitation using anti- clusterin antibodies and analyzed proteins via MALDI-TOF-MS in control and LPS-stimulated DCs from three healthy donors. Data analysis revealed 114 differentially co-precipitated proteins in LPS-treated DCs (p<0.05), with 48 showing stronger significance (p<0.01). Gene Set Enrichment Analysis indicated that clusterin interacts with proteins involved in apoptosis regulation (p<0.00091), immune response (p<3.10e-7), actin cytoskeleton organization (p<3.10e-12), and extracellular exosome processes (p<6.10e-27). These findings confirm clusterin's role in modulating DC death and suggest novel functions in DC physiology.

11. 261. **LSP1 DEFICIENCY DISRUPTS MEMORY T CELL AND B CELL RESPONSES IN MICE**

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2. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas. Universidad Nacional de Córdoba, DBC-FCQ-UNC *The Leukocyte-Specific Protein 1 (LSP1) is a 52 kDa cytoplasmic phosphoprotein expressed in leukocytes and endothelial cells in both humans and mice, demonstrating significant functional conservation across species. LSP1 interacts with F-actin, modulating the cytoskeleton and influencing cellular motility and interactions. It also contains $Ca2^+$ binding domains, acting as a substrate for multiple kinases in T lymphocytes (TL) and B lymphocytes (BL), indicating its involvement in immune signaling pathways.*

Previous studies have shown that dendritic cells (DCs) lacking LSP1 have impaired antigen uptake and processing, resulting in defective antigen presentation to $CD4^+$ and $CD8^+$ T lymphocytes. To explore LSP1's role in adaptive immunity, we conducted experiments in LSP1 knockout (KO) mice immunized with OVA/CpG-ODN 1826 in a nanoformulation. Immunized KO mice underwent a delayed hypersensitivity reaction test (mainly mediated by $CD4^+$ TL) through injection of OVA into plantar foot pad. Also, after challenge with OVA/CpG-ODN 1826, cellular suspensions from various organs of immunized mice were analyzed by flow cytometry to examine T and B lymphocyte responses. Our findings revealed that LSP1 KO mice showed reduced edema at the OVA inoculation site compared to wild-type (WT) mice, suggesting a diminished inflammatory response. Moreover, KO mice failed to induce OVA-specific central memory and effector memory $CD8^+$ T lymphocytes. In terms of B lymphocytes, LSP1 KO mice generated lower levels of OVA-specific IgM and IgG B cells than WT mice. Despite this, the total IgG and IgG1 and IgG2c levels were comparable between the two groups. Notably, bone marrow of LSP1 KO mice contain lower percentages of $CD138^+$ antibody- producing cells, indicating that LSP1 may regulate B cell function. In conclusion, LSP1 KO mice exhibit impaired $CD8^+$ T lymphocyte memory responses and reduced generation of specific B cells and APCs, maintaining similar antibody levels to WT mice.

12. 298. **ROLE OF TRANSCRPTIONAL CO-FACTOR IRF2BP2 IN ACTIVATION AND HYPORESPONSIVENESS OF CD8 T CELLS**

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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 $CD8^+CD44^+$ popu-

lation and an increase of granzyme B in effector cultures. In memory culture, cells from IRF2BP2 TKi animals revealed an increase of IFN- gamma 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.

13. 337. **T CELL MODULATION OF CD73+ CORD BLOOD PLASMA EXTRACELLULAR VESICLES**

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Background: CD39 and CD73 are ectonucleoatidases that can hydrolyze extracellular ATP yielding AMP and adenosine. Newborns have elevated plasma adenosine levels, which negatively regulate immune response. Human umbilical cord blood (CB) cell-derived extracellular vesicles can play immunosuppressive roles; however, plasma-derived extracellular vesicles (CB pEVs) remain poorly studied. Objetives: To isolate and characterize the CB pEVs to confirm the expression of CD73. To assess the ability of these CB pEVs to hydrolyzed ATP and produce adenosine, and to investigate the impact of adenosine production by CB pEVs on T cell function. Methods: CB pEVs were isolated using size exclusion chromatography. Characterization included western blot, bead-based flow cytometry, nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). ATP levels were measured by luminometry while adenosine levels by fluorometry. The effects of T cells exposed to CB pEVs were analyzed by flow cytometry. Results: Isolated CB pEVs displayed typical EV characteristics, including a cup-shaped appearance in TEM (n=1), enrichment of EV markers CD9, CD81, and ALIX (n=4), and a size distribution of 131± 65nm (n=3). Importantly, we established the expression of CD39 (n=4, flow cytometry) and CD73 (n=3, western blot) in CB pEVs. We also found that CB pEVs exhibited potent and dose-dependent ATP hydrolytic activity (n=6), which was partially inhibited by ARL, an ecto-ATPase inhibitor ($p<0.01$). Additionally, they demonstrated the capacity to generate adenosine (5±2µM, n=6). Furthermore, CB pEV exposure significantly decreased T cell proliferation, while it did not induce apoptosis. Interestingly, they increased the proportion of Treg cells ($p<0.03$, n=6). Conclusion**:** CB pEVs could perform both hydrolytic steps sequentially to form adenosine from ATP. Their ability to generate the anti-inflammatory adenosine, and modulate T cell function highlights their potential for therapeutic applications in immune regulation.

14. 368. **CD4+ T HELPER CELL SUBSETS IN MAJOR DEPRESSIVE EPISODE: LONGITUDINAL STUDY**

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Background. Depression is a highly prevalent psychiatric condition referred to as a Major Depressive Episode (MDE). Inflammation has been

proposed to contribute to the clinical scenario with altered innate and adaptive immune responses. We previously reported a cross-sectional study showing an increased inflammatory response with unbalanced monocytes and $CD4^+$ T cell activation in MDE; however, the follow-up course of the immune response was not explored. **Objective.** We aimed to evaluate the effector $CD4^+$ T helper (Th) subsets cytokines and C-reactive protein (CRP) changes in the blood of MDE patients at baseline and 6 to 12 months follow-up.
Methods. We examined 36 patients (aged 18-65) with a diagnosis of MDE, including 19 with active and 17 with the remitted condition, defined by the 17-item Hamilton Depression Rating Scale (HDRS-17) at baseline and follow-up. $CD4^+$ effector cells were analyzed in peripheral blood mononuclear cells by intracellular production of $IFN\gamma^+$ (Th1), $IL\text{-}17^+$ (Th17), and $CD4^+IL\text{-}10^+$ cells by flow cytometry. Plasma cytokine levels were measured using a bead-multiplex assay and CRP using an immunoturbidimetric test. **Results.** HDRS-17 evaluation showed that remitted patients did not change their condition; however, patients with active MDE showed a significant reduction at the follow-up compared to baseline ($p<0.009$). Interestingly, patients with active MDE showed a reduction in the frequencies of Th1 ($p<0.0001$), Th17 ($p<0.0001$), $CD4^+IL\text{-}10^+$ ($p<0.0001$), $IN\text{-}F\gamma+IL\text{-}17^+$ ($p<0.023$) and $INF\gamma^+IL\text{-}10^+$ ($p<0.018$) between baseline and follow-up. Among the cytokines evaluated, we found that the soluble triggering receptor expressed on myeloid cells-2 (sTREM-2) significantly reduced its level at the follow-up compared with the baseline in active patients ($p<0.036$). Surprisingly, CRP levels showed a moderate increase in the follow-up of active patients ($p<0.013$). **Conclusion.** These results indicated that effector $CD4^+$ T cells are critically associated with the active condition during the disease course. Nonetheless, CRP levels suggest a continuous low-grade inflammation.

15. 373. **DECODING THE ROLE OF PERHEXILINE MALEATE IN ENHANCING DENDRITIC CELL-MEDIATED ANTIGEN CROSS-PRESENTATION**

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The immune response mediated by cytotoxic CD8+ T cells (CTLs) is crucial for both tumor immunotherapy and defense against intracellular pathogens. Dendritic cells (DCs) can internalize and present exogenous antigens (Ag) via MHC I to activate naïve CD8+ T cells, a process known as cross-presentation. Adjuvants are essential for a robust CTL response, particularly in subunit vaccines. However, due to the complexity of Ag cross-presentation pathways, identifying therapeutic targets for adjuvants that enhance protective CTL responses has been challenging. To address this, we conducted a high-throughput screening of drug libraries approved by international agencies to identify novel compounds that enhance Ag cross-presentation in DCs. Our approach involved the adaptation of the B3Z presentation assay using the JAWSII DC cell line and Ovalbumin (OVA) as the soluble Ag.
In this study, we investigate how Perhexiline Maleate (PM), one of the active compounds identified, enhances cross-presentation. While DCs generally reduce proteolytic activity in endo/lysosomal compartments to preserve Ags, PM does not affect endosomal degradation of OVA in DCs. Instead, PM increases reactive oxygen species (ROS) and lipid peroxidation, potentially causing membrane rupture, which could explain the observed increase in Ag translocation and facilitate cross-presentation. PM also inhibits the mitochondrial enzyme carnitine palmitoyltransferase 1 (CPT1), reducing fatty acid metabolism. Our previous research indicated that PM increases lipid bodies (LB) per cell, which correlates with enhanced Ag cross-presentation by DCs. Notably, this increased cross-presentation and LB accumulation do not depend on lipids in the culture medium. Additionally, DCs stimulated with PM in vitro generated a specific cytotoxic response mediated by CD8+ T cells in vivo. These findings highlight the potential of PM in immunotherapeutic strategies, with significant implications for developing targeted therapies against various diseases. Further studies will aim to clarify the intracellular mechanisms underlying enhanced cross-presentation in PM-stimulated DCs.

16. 381. **IMPACT OF CD43 ABSENCE ON ERYTHROCYTE DIFFERENTIATION AND T-CELL ACTIVATION**

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CD43 sialomucin (leukosialin, sialophorin) is a transmembrane glycoprotein expressed on all hematopoietic cells. During development, early progenitors committed to hematopoietic development express CD43. In leukocytes, we have shown that this abundantly expressed co-receptor molecule transduces signals through its cytoplasmic domains and participates in adhesion and activation. Within the erythroid compartment, CD43 is expressed in all differentiation stages except mature erythrocytes. To determine whether the absence of CD43 affected erythrocyte differentiation and T lymphocyte activation, we compared the peripheral blood hematological profiles, the erythroblast maturation by differential expression of CD44 and TER119 in the bone marrow (BM), and T-cell proliferation and viability in response to PMA/ionomycin or CD3/CD28 of WT and CD43KO male C57BL/6 mice. CD-43KO mice showed increases in hemoglobin, hematocrit, and erythrocyte size, reduced RBC levels in the spleen and blood, and increased erythroblast population in the BM compared to WT mice. Regarding T-cell proliferation, the lack of CD43 differentially impacted CD4+ and CD8+ lymphocytes. While CD43KO CD8+ T cell viability dropped abruptly at 48h post-activation, and CD4+ T cells exhibited increased proliferation, at 72h, CD8+ T cell proliferation increased dramatically, but CD4+ T cell proliferation and viability fell sharply. Thus, the absence of CD43 impairs erythroblast differentiation, reducing RBC levels in the blood, with the increased hematological profiles possibly compensating for decreased RBCs in CD43 KO mice. In T lymphocytes, CD43 appears to function as a negative regulator in CD8+ T cells and a positive regulator in CD4+ T cells. Altogether, our data highlight novel functions for CD43.

17. 387. **DIABETES MEDIATED IMPAIRMENT OF CD8+ T-CELL RESPONSE IN TRYPANOSOMA CRUZI INFECTION**

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Type 2 diabetes mellitus (T2DM) involves chronic low-grade inflammation, associated with increased infection risk. We investigated how T2DM impacts CD8 T-cell function in *Trypanosoma cruzi* infection model and compared these results with PBMCs from T2DM patients. To this aim, C57BL/6 mice were fed with 34.5%Kcal fat diet and 20% fructose in drinking water for 20 weeks. At week 8, a single dose of streptozotocin (100 mg/kg) was intraperitoneally injected to establish experimental diabetes (D+T). As expected, D+T group exhibited increased glycemia compared to control group (N-standard diet) ($p=0.01$). Diabetic (D+Ti) and non-diabetic (Ni) mice were infected with 1000 trypomastigotes. For patient studies, PBMCs from 10 T2DM and 10 non-diabetic individuals were analyzed. D+Ti group showed reduced frequency of circulating ($p=0.0094$) and splenic ($p=0.0146$) granzyme B+CD44+CD8+ T-cells. D+Ti also evidenced higher frequency of circulating IFN-g+CD44+CD8+ T-cells ($p=0.0118$) and lower frequency of TNF-a+CD44+CD8+ T-cells ($p=0.0110$), with the opposite pattern in the spleen. Additionally, higher frequency of circulating CD8 T-cells with mitochondrial depolarization ($p=0.0190$) was observed in D+Ti group. Splenic CD8 T-cells from D+T showed higher lipid droplet (LD) content compared to controls (D+T=31.1±11.1%, N= 1.3±1.3%; $p<0.0001$). Strikingly, LD were absent in these cells from D+Ti group. To evaluate the impact on functionality, D+Ti or Ni CD8 T-cells were co-cultured with in vitro infected macrophages. Decreased frequency of apoptotic macrophages ($p=0.0061$) and higher parasite number/cell ($p=0.0289$) was found with T-cells from diabetic vs. from controls. Accordingly, CD8 T-cells from T2DM PBMCs showed lower frequency of granzyme B+ ($p=0.0495$) and higher frequency of IFN-g+CD8+ T-cells ($p=0.0296$) compared to non-diabetic controls following PMA-ionomycin stimulation. Moreover, T2DM patients showed higher frequency of CD8 T-cells with depolarized mitochondria ($p=0.0438$) and LD content ($p=0.0474$) than controls. Although further research is needed, our preliminary results suggest that DMT2 impairs CD8 T-cell effector profile, possibility by inducing lipid metabolism alterations.

18. 487. **THYROID STATUS MODULATES SPLENIC DENDRITIC CELL SUBSETS IN MICE**

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Background: Thyroid hormones (THs) influence several aspects of innate and adaptive immunity. Physiological concentrations of triiodothyronine (T3), the active TH, stimulate dendritic cell (DC) maturation and enhance their capacity to promote proinflammatory and cytotoxic adaptive immune responses while restraining regulatory signals. Additionally, T3 improves the antitumor functions of DCs in melanoma and colon carcinoma models.
Objectives: This study aimed to investigate the impact of *in vivo* thyroid status on DCs, considering that hypothyroidism and hyperthyroidism are common endocrine disorders worldwide, and mainly of autoimmune origin.

Methods: C57BL/6 mice were rendered hypo- or hyperthyroid by administering propylthiouracil (0.5mg/ml) for 18 days or T4 (0.012mg/ml) for 28 days in drinking water, respectively. Control animals received plain water. Plasma TH levels were measured using immunoassays. Pituitary TSHβ mRNA expression was determined by qPCR. Hepatic leukocyte (CD45+) infiltration and splenic DC subsets were analyzed by FACS, including plasmacytoid DCs (pDCs:CD19-B220+) and conventional DCs (cDCs:MHCII+CD11c+), focusing on cDC1 (CD8a+) and cDC2 (CD11b+) subpopulations. Statistical analysis: One-way ANOVA-Tukey's test ($p<0.05$). Results: Hypothyroid mice showed reduced TH levels, while hyperthyroid mice had elevated TH levels (vs control animals, $p<0.0001$). Consistently, TSHβ mRNA expression was significantly increased in hypothyroid and decreased in hyperthyroid mice. A significant increase in leukocyte frequency was registered in the liver of hyperthyroid mice, in contrast to hypothyroid and control animals. Hyperthyroid mice exhibited pronounced splenomegaly, and a significant increase in the frequency of splenic pDCs and cDCs within the CD45+ cell population compared to both control and hypothyroid groups. This result included a notable rise in both cDC1 and cDC2 subsets. Conclusion: Hyperthyroidism induces splenomegaly, and increases the number of DCs and their subsets. These findings encourage further research on DC maturation and function in the context of thyroid disorders, to uncover the mechanisms involved in the immune disorders resulting from thyroid pathology.

19. 544. **EXPLORING DP16(1)/YEY MOUSE MODEL TO UNDERSTAND THE ENHANCED SUSCEPTIBILITY OF CHILDREN WITH TRISOMY 21 TO RESPIRATORY TRACT INFECTIONS: IMPACT OF A VIRAL DOUBLE-STRANDED RNA MIMETIC ON LUNG IMMUNE CELL POPULATIONS**

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Background: Children with Trisomy 21 (T21) are at a higher risk of contracting respiratory tract infections such as respiratory syncytial virus and more likely to require hospitalization with longer hospital stays and more probability of requiring ventilatory support and intensive care. Objectives: To evaluate the impact of poly IC, a double stranded RNA viral mimetic on the immune populations present in the lungs of Dp16(1)/Yey (DP16), a mouse model of T21. Methods: DP16 and control mice (n=12) were i.p. injected with poly IC and 15hs later, sacrificed. A 29-parameter spectral flow cytometry was used to characterize immune cells within spleen, lung, inguinal lymph nodes (iLN) and mesenteric lymph nodes (mLN). Results: At basal levels, most immune populations present in lungs of DP16 mice showed a similar distribution than controls, except for a higher ratio of CD4+/CD8+ T cells and a significant reduction in natural killer cells (NK) ($p<0.05$). In contrast, upon poly IC stimulation the total number of leukocytes (CD45+ cells) diminishes in DP16 mice ($p<0.05$), and a huge remodeling of the immune compartment occurs: the frequency of B cells expressing activation markers such as CD69 augments ($p<0.0001$), indicating an hyper response in lungs of DP16 mice. Interestingly, among mature NK cells, the proportion of them expressing the chemokine receptor Cx3cr1 also significantly rises in DP16 mice ($p<0.001$). Of note, when overexpressed, Cx3cr1 chemokine

receptor contributes to chronic inflammation in lungs. Conclusions: These results suggest that in the DP16 lung, T, B and NK cells hyper respond to poly IC stimulus compared to controls, which could explain the exaggerated response to viral infections seen in children with T21.

20. 581. **UNDERSTANDING THE IMMUNE DYSREGULATION IN CHILDREN WITH DOWN SYNDROME: ARE CXCR3+ T FOLLICULAR HELPER CELLS INVOLVED IN THE AUTOIMMUNE-PRONE PROFILE REPORTED IN THESE POPULATION?**

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Children with Trisomy 21 (T21) have an elevated risk of respiratory infections and distinctive susceptibility to diseases, which arise partially from an early immune dysregulation. We investigated T-B cell interactions in tonsils, as a model of secondary lymphoid organs (SLO). We looked for the germinal center (GC) reaction, a specialized area within follicles of SLOs where mature B cells undergo intense proliferation, differentiation, and selection during an immune response, with the help of specialized CD4+T cell subsets: the follicular helper T cells (Tfh). Methods: Multiparametric flow cytometry and multiplex immunofluorescence characterized B and T cells in hypertrophied, non-infected tonsils in T21 children compared to age-matched controls (n=10). We focused on the expression of CXCR3 and other markers associated with Th1 response, inflammation and autoimmunity. The architecture and frequency of different populations from the tonsils' B and T zones were analyzed. Results: There was a marked remodeling of T-B cell compartments in T21 tonsils, with increased frequency of different CXCR3-expressing cells, such as memory B cells ($p<0.05$), GC B cells, plasmablasts ($p<0.01$) and activated non Tregs nor Tfh CXCR3+ T cells in detriment of conventional Tfh ($p<0.05$). The density (#cells/mm2) of CXCR3+ Tfh-like cells outside follicles significantly increased in T21, suggesting heightened extrafollicular responses. CXCR3+ Tfh and B cells were closer together in the T zone of T21 tonsils, suggesting stronger interactions outside follicles. Conclusions: T21 substantially impacted the tonsil T and B cell compartments, inhibiting Tfh and GC formation while increasing CXCR3+ B cells in the T cell zone. Our results suggest that, not only the GC reaction is altered, but the spatial distribution of T and B cells is modified in T21 tonsils.

21. 588. **P7F8, A HUMAN ANTIBODY AGAINTS SARS-COV-2 VARIANTS OF CONCERN-P7F8, A HUMAN ANTIBODY AGAINTS SARS-COV-2 VARIANTS OF CONCERN**

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The development of vaccines against COVID-19 as a first line of defence was a major breakthrough in reducing the incidence of the disease and has provided protection against variants of the virus with an enhanced immune response. The study of the humoral immune response through the isolation and characterisation of specific antibodies against the virus from convalescent and/or vaccinated patients will allow us to know the evolution of these antibodies against future reinfections. The aim of this work was to select and characterise a group of human monoclonal antibodies against the RBD of SARS-CoV-2, capable of inhibiting the interaction of protein S with the human ACE-2 receptor. Using the Epstein-Barr virus memory B-cell immortalisation method, we selected whole blood samples from confirmed

and convalescent volunteers with disease that had antibodies with recognition of the RBD domain of the SARS-CoV-2 S protein. From venous blood PBMC and by MACS and FACS selection, the number of RBD-specific antibody-producing cells was quantified and immortalised. Once the lymphoblastoid cell lines were developed, the best antibody-producing clones were characterised and determined by recognition assays. The P7F8 antibody is able to recognise the SARS-CoV-2 RBD with an affinity for: WT of 0.009nM, Beta 0.018nM, Delta 0.051nM, Omicron BA.4/BA.5 0.148nM, Omicron BA.1 0.880nM, Omicron XBB 2.607nM and Omicron BQ.1.1 0.065nM. Its neutralising capacity was tested in vitro, exhibiting an IC50 of 1.79ug/mL for D614G, 5.4ug/mL for Delta and 0.47ug/mL for Ómicron BA.1. After sequential analysis, the antibody shows a previously described structural motif with cross-reactivity against other Sarbecoviruses (Liu et al., 2022), which confers broad neutralisation not only against SARS-CoV-2 variants, but also against SARS-CoV with an affinity of 2.056nM.

22. 590. **BISPECIFIC ANTIBODIES AGAINST THE MAIN SARS-CoV-2 VARIANTS OF CONCERN: AN EFFECTIVE STRATEGY**

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COVID-19 is a significant concern due to the continuous evolution of SARS-CoV-2 and its mutagenic potential to generate variants that may escape the immune response, which potentially diminishing the neutralizing efficacy of antibodies. Our objective is to develop bispecific antibodies derived from neutralizing monoclonal antibodies that recognize distinct epitopes from RBD of SARS-CoV-2, These antibodies were obtained from B-cells from convalescent Mexican patients, who have recovered from different SARS-CoV-2 variants. The B-cells were immortalized using Epstein-Barr virus and expanded as lymphoblastoid cell lines. Four monoclonal antibodies were selected, which belong to different germline lines and exhibit relatively low IC50 values. RNA was extracted and converted to cDNA via RT-PCR. The variable regions of the heavy and light chains were then amplified by PCR. To construct bispecific antibodies, we use the single chain Fragment antibody (scFv) format (VH-VL) from each monoclonal antibody to generate the scFv1-(G4S)4-scFv2-Fc IgG1 format (Chiyyeadu et al., 2024). Previously, we ensured that the antibodies selected do not compete. We generate two bispecific antibodies S56G6 y P7S57. It was noted that the orientation of the antibodies is critical for effective recognition, for that reason both VH-VL and VL-VH were tested. Using overlapping PCR, we assembled the orientation within the pFUSEss-CHIg-hG1 vector, which was transiently expressed in EXPI 293 cells and subsequently purified via affinity chromatography. The multivalent binding to the RBD resulted in increased affinity and neutralizing activity against various SARS-CoV-2 variants compared to the monoclonal antibody and an additive effect of its properties. This improvement is attributed to multivalent interactions within the same antigen, which likely results in the formation of a two-epitopes-antibody complex in accordance with what was previously reported (Wang et al., 2024). Thus, bispecific antibodies represent a promising strategy against SARS-CoV-2.

23. 596. **HELIOS REGULATES CD8 T CELL EFFECTOR DIFFERENTIATION**

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Helios (*Ikzf2*) belongs to a family of transcription factors that regulate lymphoid differentiation and function. In thymic-derived FoxP3 regulatory T cells, Helios promotes suppressive function. Previously, we observed that Helios is induced in CD8 T cells rendered anergic.
The aim of this work was to analyze Helios induction and function in CD8 T cells. We mounted an *in vitro* assay to identify factors that modulate Helios expression. Productive CD8 activation, achieved by high affinity ligands and pre-activation of antigen presenting cells with LPS, inhibited Helios expression. In contrast, activation with low-affinity ligands led to robust Helios induction. Pharmacological dissection of signaling pathways indicated that activation of the IL-2-STAT5 pathway inhibited Helios induction. We analyzed Helios expression kinetics in CD8 T cells during an acute infection, where IL-2 is important. We observed an increase in Helios expression that peaked at 72 hours post infection, however this expression was transitory and did not persist. *Ikzf2*-deficient OT-I cells exhibited impaired proliferation and expansion when exposed to OVA associated with *Listeria*, similar results were found using P14 CD8 T cells exposed to LCMV-Arm infection. OT-I deficient cells produced less IFN-g than control cells. scRNA sequencing revealed that Helios deficient cells resemble memory/progenitor cells as expression of *Tcf7*, *Bach2*, *Il7r* was upregulated. Also, we found that Helios downregulated genes involved with oxidative phosphorylation. In summary, Helios fine-tunes proliferation and effector differentiation during CD8 T cell activation. IL-2-STAT5 activation inhibits Helios expression during strong CD8 stimulation. Because Helios is induced by suboptimal TCR engagement, it could regulate the activation of lower affinity clones during immune responses.

24. 715. **TGFB RECEPTOR 3 (BETAGLYCAN) PROMOTES IN VITRO INDUCTION OF REGULATORY T CELLS WITH ENHANCED SUPPRESSIVE PHENOTYPE**

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TGFBR3 or betaglycan is a widely expressed glycoprotein that facilitates high-affinity binding of several ligands of the TGF-β superfamily to TGFBR1 and TGFBR2. Betaglycan has been described as an important regulator of fetal development, reproduction, tumor suppression, and T cell maturation and effector differentiation, but its role in the regulation of immune responses remains poorly understood. Our group has previously reported that expression of TGFBR3 is downregulated during iTreg induction and that blocking of TGFBR3 reduces iTreg conversion. Since TGF-β signaling is crucial for the induction and suppressive function of regulatory T cells (Treg), we sought to determine if betaglycan could be crucial to these processes *in vivo.* Due to the embryonic lethality of betaglycan knockout mice, we generated a conditional knock-out mouse model in which betaglycan expression is abrogated in peripheral mature T cells by using the distal promoter of Lck (Tgfbr3$^{fl/fl}$.dLcKCre). Naïve CD4+ CD25- CD62L+ CD44 low T cells were FACSsorted and cultured in the presence of anti-CD3/CD28 Dynabeads and TGF-β1 (1ng/ml) and iTreg induction was evaluated at 3 days after stimulation. Interestingly, iTreg induction was 20% lower in Tgfbr3$^{fl/fl}$.dLckCre cells compared to Tgfbr3$^{fl/fl}$ cells (0.80 RI %CD25+ FoxP3+, p=0.0079, Mann-Whitney test). Since we also observed a reduction in FoxP3 expression (0.93 RI MFI FoxP3, p=0.0286, Mann-Whitney test) in TGFBR3 deficient iTreg, we are currently investigating whether their suppressive function might be compromised. Our data show that betaglycan supports iTreg induction and could potentially favor their suppressive function which plays an important role in the regulation of immune responses in various diseases.

ALLERGY

25. 134. **ANTI-INFLAMMATORY EFFECT OF AGAVE FRUCTANS (AGAVE TEQUILANA WEBER VAR. AZUL) IN A RAT MODEL OF ATOPIC DERMATITIS**

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Atopic dermatitis (AD) is a cutaneous allergic disease characterized by pruritus, inflammatory

lesions, and *Staphylococcus aureus* colonization in the skin. Agave fructans are not digested by human enzymes but are metabolized by intestinal microbiota producing substances with anti-inflammatory activity at gut and distal tissues. In this study, we examined the effect of fructans from *Agave tequilana* Weber var. azul on skin inflammation, pruritus and *S. aureus* colonization in rats. AD was induced in the ear of the animals and then fructans (0.1, 1 or 5 g/kg) were orally administered for 13 days. After ear allergen application, tissue swelling was evaluated and scratching events counted. The concentration of IgE in serum was analyzed by ELISA. Slides from lesional tissue were stained to evaluate mast cells hyperplasia and epidermal thickness. *Staphylococcus epidermidis* and *S. aureus* were quantified in the skin by PCR. Data were analyzed by ANOVA with Dunnett's test, $p < 0.05$. Treatment with fructans at 0.1g/kg resulted in a significant decrease of ear thickness induced 1 and 6 h after allergen application, with no change at 24 h. Likewise, the increase of ear weight produced 1 h after allergen challenge was reduced. The lowest fructans dose reduced serum IgE levels to control values. Dermal mast cell infiltration and epidermal thickening were significantly decreased with all fructans doses, although the effect was greater at 0.1g/kg. Scratching events were slightly increased or remain unchanged. The amount of *S. aureus* in the lesions was decreased after treatment with fructans, and *S. epidermidis* levels were restored to control values. In conclusion, intake of agave fructans, mainly at 0.1g/kg, reduces the inflammatory response and *S. aureus* colonization in the skin in a model of AD; although a dose-response effect was not observed. Agave fructans effect on pruritus requires further investigation.

26. 231. **TOXOPLASMA GONDII SERINE PROTEASE INHIBITOR-1 (RTGPI-1) HAS THE ABILITY TO MODULATE THE ACTIVATION OF ALLERGEN-SPECIFIC TH2 LYMPHOCYTES WITHOUT GENERATING CHANGES IN THE TH1 RESPONSE**

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Previously, we demonstrated that intranasal administration of rTgPI-1 with OVA relieved asthma symptoms and reduced lung cDC2 population without differences in cDC1 subset. Also, BMDC exposed to rTgPI-1 showed lower capacity to activate Th2 and Th17 cells. We aimed to study the correlation between the dendritic cell populations with changes in the resulting adaptive T cell response in the lung, and to analyze whether the effect of rTgPI-1 can be reproduced in peripheral blood mononuclear cells (PBMC) from allergic patients. BALB/c mice were sensitized with OVA/Alum, challenged with aerosolized OVA and intranasally treated with rTgPI-1+OVA. Lung cells were *ex vivo* stimulated with OVA and cytokine profile was analyzed. PBMC from patients with rhinitis and/or allergic asthma and with a positive prick test to *D. pteronyssinus* were *in vitro* stimulated with House Dust Mite (HDM) with or without rTgPI-1. Allergic mice showed higher IL-4 and IL-5 production than *naive* animals ($p<0.0001$), which was reversed with intranasal rTgPI-1 treatment. A significant decrease in antigen-specific IL-4 ($p<0.01$) and a trend to diminished IL-5 was observed. No significant changes in IFN-γ, IL-17 and IL-10 were detected (ANOVA/Tukey). Similarly, PBMCs from allergic patients stimulated with House Dust Mite (HDM) in the presence of rTgPI-1 showed decreased IL-4 and IL-5 levels ($p<0.05$), with no significant changes in IFN-γ and a trend towards reduced IL-17 (Wilcoxon test). The cytokine secretion profile in the lungs of rTgPI-1 treated mice in response to the allergen correlates with the previously observed reduction in the cDC2 subset. Moreover, the results from PBMCs of allergic patients align with the lung cell culture cytokine profile, indicating that rTgPI-1 can modulate the activation of allergen-specific Th2 lymphocytes without affecting the Th1 response. These results support further preclinical studies aimed at advancing allergen-specific immunotherapy.

27. 232. **MODULATION OF ATOPIC DERMATITIS BY ADMINISTRATION OF TOXOPLASMA GONDII TOTAL LYSATE ANTIGENS**

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BACKGROUND: Atopic dermatitis (AD) is a chronic inflammatory skin disease driven by a type 2 immune response. Developing therapies that are both effective and safe remains challenging. Previously we demonstrated that Toxoplasma gondii (T. gondii) infection reduces AD development. We hypothesize that immunomodulatory molecules from T. gondii may contribute to this protective effect. OBJECTIVES: To study the ability of T. gondii total lysate antigens (TLA) to modulate AD in a murine model. MATERIALS AND METHODS: BALB/c mice were sensitized and challenged with ovalbumin (OVA), then treated with Calcipotriol and OVA patches. TLA was administered intradermally to sensitized mice (TDA group), while the control group (DA) received PBS. Negative controls (N) received ip, sc, and epicutaneous applications with PBS and were not exposed to Calcipotriol. Allergen-specific antibody levels, skin histopathology, and cytokine balance were evaluated. RESULTS: No significant difference was observed in mast cell infiltration or in the thickness of the epidermis and the keratin layer between TDA and DA mice. However, a clear trend towards lower eosinophil levels and an increase in neutrophil infiltration ($p<0.05$) in the dermis were detected in TDA mice. TLA administration also showed a tendency to reduce IgE levels and increase IgG2a antibodies. The changes in IgE levels and eosinophil skin infiltration were such that they did not significantly differ from those in the N mice. IL-4 and IL-5 secretion levels showed no significant differences between DA and TDA mice. However, an increase in IFN-γ was observed in TDA compared to the DA group ($p<0.05$). CONCLUSION: These findings suggest an improvement in the pathology and a potential shift towards a type 1 immune response. Although further research is needed, these results could contribute to the development of strategies aiming to modulate atopic dermatitis.

28. 240. **IDENTIFICATION OF PERIPHERAL BLOOD AND COLONIC LAMINA PROPRIA IGE + MEMORY B CELLS**

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Given the rising incidence and severity of allergic manifestations and the critical role of IgE as a therapeutic target, it is imperative to investigate the mechanisms underlying the synthesis, secretion, and regulation of IgE. Our group studied colorectal juvenile polyps (JP) from atopic pediatric patients with proctorrhagia received at the Children's Hospital of La Plata. These patients show elevated total and specific IgE to food allergens in peripheral blood (PB) and JP, with type 2 inflammatory infiltrate dominated by IgE-producing plasma cells and sensitized eosinophils. JP showed active germinal centers where IgE is synthesized. There is no in vivo evidence of IgE+ memory B cells (LBmem) generation in humans. We propose that the inflamed colonic mucosa of allergic patients is a site of differentiation of IgE+ memory B cells. This work aims to detect IgE+ memory B cells in polyps of food allergen-sensitized pediatric patients. Our work involved a comprehensive analysis of PB and JP from IgE-sensitized patients by flow cytometry using a range of antibodies including a polyclonal (IgE Bv711) and a monoclonal (Omalizumab A488) anti-IgE in a panel of antibodies (a-CD45 PerCP, a-CD19 V500, a-CD27 APC, a-CD138 PE, a-IgD SB600).

We identified IgE+ memory B cells, with the monoclonal antibody, in 4 JPs (1,12%, 2,94%, 2,97%, and 5,05%) and their respective PB (0,04%, 1,36%, 5,62% and 0,39%), while the polyclonal anti-IgE antibody was useful for IgE+ cell quantification in PJs(1,09%, 5,67%, 16,96%, and 13,64%) and PB (0,10%, 1,91%, 16,02% and 2,36%).

In conclusion, we detected IgE+ memory B cells in human colonic tissue, being essential to continue delving deeper into the pathology and severity of IgE-mediated inflammatory disorders related to food antigens.

29. 465. **CHARACTERIZATION OF TYPE 2 INFLAMMATORY RESPONSE OF COLONIC AND ESOPHAGEAL MUCOSA AND STUDY OF INTERACTION BETWEEN FIBROBLASTS, EOSINOPHILS, AND INTESTINAL EPITHELIAL CELLS IN A MODEL OF GASTROINTESTINAL EOSINOPHILIC DISEASE**

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Eosinophilic gastrointestinal diseases (EoGD) are inflammatory pathologies characterized by the recruitment of eosinophils to the lamina propria and fibrosis. Eosinophilic esophagitis (EoE) is the EoGD with the highest incidence. However, underlying molecular mechanisms remain unclear. We have previously characterized colorectal polyps from pediatric patients (CP) sensitized to food allergens with a similar tissue inflammatory context. This study aims to evaluate the colonic and esophageal mucosal response induced by type 2 inflammation and the cellular and molecular interactions between intestinal fibroblasts, eosinophils, and epithelium from CP and EoE. Caco-2 and EPC-2 cells were stimulated with rh-IL-13, rh-IL-4, or rh-IFN-γ, and CCL26 was measured in culture supernatants. Eosinophils from CP were sorted and cultured. CP fibroblasts were also cultured and characterized. IL-8 and CCL26 were assessed after stimulation with rh-IL-13, rh-IL-4, rh-IL-9, rh-TGF-β, and eosinophil-conditioned media. EoE biopsies and CP were studied using immunofluorescence staining for histological characterization and IgE detection. The esophageal biopsies response to type 2 cytokines was examined *ex-vivo*. Type 2 cytokines induced CCL26 secretion on colonic (IL-13: 128,2+/-22,3 ($p<0,0001$); IL-4: 196,0+/-23,2 vs. medium: 51,7+/-31,2pg/ml) and esophagus (IL-13: 135,6+/-7,9 ($p<0,01$); IL-4: 160,8+/-10,0 vs. medium: 94,8+/-3,0pg/ml) epithelial cells and CP fibroblasts (IL-13: 114,1+/-61,3; IL-4: 147,2+/-114,0 vs. medium: 36,6+/-8,7pg/ml). CP fibroblast expressed vimentin and secreted IL-8 under Th2 stimuli (IL-9: 523,4+/-211,1 vs. medium: 482,3+/-45,0pg/ml), and eosinophil-conditioned media (172,4 vs. medium: 57,6pg/ml). Mucosal sections of EoE biopsies and CP expressed TSLP, CCL26, and α-SMA (an activation fibroblast marker). Moreover, IgE was expressed in CP but not in EoE biopsies. Increased CCL26 (IL-13: 210,2+/-295,3 vs. medium: 132,2+/-97,8pg/ml) and TSLP (IL-13: 96,3+/-91,7 ($p<0,01$) vs. medium: 26,7+/-23,5pg/ml) secretion was observed in esophagus biopsies following *ex-vivo* stimuli. In conclusion, we showed that epithelial cells and fibroblasts secrete chemokines and alarmins in the presence of type 2 cytokines, which could drive the recruitment of eosinophils in EoGD.

30. 476. PROBIOTICS AND RESPIRATORY ALLERGY: UNCOVERING THE ROLE OF MEMORY B CELLS IN A MOUSE MODEL

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Commercial probiotic fermented-milk (PFMc) administered continuously to ovalbumin (OVA)-sensitized mice effectively modulated the allergic response. This treatment reduced levels of a-OVA-IgE and IL-4, while promoting a Th1 response by increasing a-OVA-IgG2a, IFN-γ, and IL-12 compared to untreated mice. This modulation was mediated by gut regulatory dendritic cells, shifting the response from a typical Th2-driven allergic reaction. It has been theorized that recurrence of allergic responses is linked to generation of immunological memory. Currently we aim to investigate if there are memory B cells forming during OVA sensitization and whether the intake of PFM has influence in their establishment. Six weeks old BALB/c mice were sensitized following three subcutaneous OVA injections (0.05 ml 1%-PBS) plus six days with OVA aerosols (20min/day) and restimulation 7-days later (sensitization-control-"SC"); a group of sensitized animals received continuously PFM containing *L. paracasei* CNCMI 1518 (PFM+OVA). 7 days-post-sensitization (7dPS) and 2 days-post-restimulation (2dPR) anti-OVA-IgE was measured in serum and bronchoalveolar-lavage (BAL); cells from Peyer's patches and lungs were cultured with or without OVA stimulation for cytokine quantification, another part was preserved to analyze IgG1 and CD138 expression (reservoir for IgE production);

results were compared to no-sensitized-mice with PFM (PFM-group) and without PFM (normal-control-group NC). A-OVA IgE increased in SC and PFM+OVA serum vs. PFM at 2dPR, no differences were found in BAL. IFN-γ secretion increased in PFM+OVA group at 7dPS and 2dPR; IL-4 showed no differences. $IgG1^+/CD138^+$ B-cells were increased in PP from PFM+OVA at 7dPS and lowered at 2dPR, while significantly increased for SC at 2dPR. PFM has a slight impact on the development of IgG1+/CD138+ B cells, but immunological memory also involves T-memory-cells. Elevated IFN-γ levels are essential for modulating Th17-population, linked to asthma severity and neutrophil infiltration in the airways. Further studies are needed to understand how IFN-γ and PFM influence immunological memory establishment.

31. 726. **EFFECT OF PROSTAGLANDIN D2 RECEPTOR ANTAGONISM ON PULMONARY ALLERGIC INFLAMMATORY RESPONSE IN MICE**

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Background: Eosinophils are granulocytes classically characterized as effector cells of the symptomatology of allergic diseases and the protection against helminths. On the other hand, important immunomodulatory and even homeostatic functions have also been attributed to eosinophils due to cytokine secretion, 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. OBJECTIVES: The aim of this study was to evaluate the role of DP2 receptor in regulating the secretion of immunomodulatory in the inflammatory allergic response in vivo in mice. Methods: A model of pulmonary allergic inflammatory reaction in baçb/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 allergic response, mice were treated with the DP2 receptor antagonist, Cay10471. And to study the role of IL-10 in the DP2 antagonism driven effects, animals were treated with Cay10471 in combination with anti-IL-10 antibody. Results: After the allergenic challenge, we observed the characteristic broncho-alveolar 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 allergic reaction, significantly, and promoted a beneficial effect on the control of pulmonary allergic inflammation, decreasing classical parameters such as broncho- constriction, 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 present the improvement promoted by Cay10471 of the allergic inflammatory parameters studied. Conclusion: 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.

AUTOIMMUNITY

32. 057. **YERBA MATE (ILEX PARAGUARIENSIS) REDUCES MULTIPLE SCLEROSIS SEVERITY BY MODULATING REGULATORY T CELL FUNCTION**

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Yerba mate (YM) is a popular infusion in Latin America, processed from the leaves and stems of Ilex paraguariensis. YM has been shown to have anti-inflammatory properties in several studies although the effect of YM in multiple sclerosis (MS) remains elusive. The aim of this work was to study the effect of YM on the development of MS by using the experimental autoimmune encephalomyelitis (EAE) mouse model, evaluating also its effect over Treg function. YM was administrated to mice daily by oral gavage for seven days prior to EAE induction and for the following 25 days after. As a control, the same treatment was done but providing the vehicle. EAE score was recorded daily, and inflammation was measured by flow cytometry and immunofluorescence in different tissues at the peak of the disease. Our results show that in this EAE model, the administration of YM delayed the onset of symptoms and reduced

the symptoms of the disease, together with a reduction in the incidence compared to the vehicle group. Reduced immune cell infiltration into the CNS and diminished myelin damage was also evident in the animals receiving YM. Protective effect of YM was independent of changes in the gut-microbiota content. Moreover, an increase in regulatory T lymphocytes (Tregs), immune cells capable of generating tolerance and decreased inflammation, was observed in EAE mice treated with YM, and improved suppressive capabilities after YM treatment were observed in vitro. In summary, we showed that YM promotes an immunosuppressive environment by modulating Treg function, reducing EAE symptoms, and suggesting that YM consumption could be a good cost/effective treatment for MS.

33. 058. **NOVEL MOLECULAR TARGET AND THERAPEUTICS TO CONTROL T CELL AUTOIMMUNITY IN TYPE 1 DIABETES**

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Background: Autoimmune diabetes involves T cell-mediated destruction of pancreatic beta cells resulting in hyperglycemia. Increased glucose flux enhances O-GlcNAcylation, an intracellular post-translational modification of nucleo-cytoplasmic proteins. We found that hyperglycemia increases O-GlcNAcylation of NF-kappaB subunit c-Rel at serine residue 350 and enhances the transcription of pro-autoimmune cytokines, IL-2, IFNG and GM-CSF in T cells. Our recent results show that the regulatory effect of c-Rel O- GlcNAcylation is gene dependent and it suppresses the transcription of forkhead box P3 (FOXP3) that controls Treg cell development and function. Objectives: The goal of this study is to identify novel mechanism promoting autoimmunity in type 1 diabetes and develop therapeutics to treat autoimmune diabetes. Based on our results, we hypothesize that pharmacological inhibition of O-GlcNAcylated c-Rel will decrease proautoimmune cytokines and increase FOXP3 expression in T cells and prove effective in alleviating autoimmunity in type 1 diabetes. Methods: We developed a novel peptoid, called OGC350, by molecular modeling and de novo synthesis, and studied its potential to bind to O-GlcNAcylated c-Rel and block its function. Results: We found that OGC350 treatment significantly decreased T cell receptor- induced, O-GlcNAcylation-dependent expression of pro-autoimmune cytokines and enhanced FOXP3 expression in T cells. OGC350 treatment selectively affected autoimmunity-associated genes and did not exhibit toxicity on survival or proliferation of T cells. Conclusion: This study reveals c-Rel S350 O-GlcNAcylation as a novel molecular mechanism inversely regulating proautoimmune gene expression and immunosuppressive FOXP3 expression in T cells with potential therapeutic implications to treat type 1 diabetes. Broad inhibition of hexosamine biosynthetic pathway or NF- kappaB will cause many side effects due to their ubiquitous importance in multiple biological functions. Therefore, inhibitors of O-GlcNAcylated NF-kappaB c-Rel function may prove long-sought-after specific molecular therapeutic to diminish autoimmunity in type 1 diabetes.

34. 065. **MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS) PROMOTE AN INFLAMMATORY PROFILE POSITIVELY CORRELATED TO ACTIVATED CD4+ T CELLS IN A SYSTEMIC LUPUS ERYTHEMATOSUS MURINE MODEL.**

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Systemic lupus erythematosus (SLE) is a potentially fatal chronic autoimmune disease that can affect various body tissues. Both lymphoid and myeloid cells play an essential role in the development and progression of the pathology. In recent years, myeloid-derived suppressor cells (MDSCs) have been increasing their impact in the study of chronic diseases. These cells correspond to a heterogeneous population characterized by a monocytic (M-MDSCs) and granulocytic (G-MDSCs) profile. In SLE, these cells do

not have a well-defined role since they have been observed to induce both pro- and anti-inflammatory responses on CD4+ T cells. In another chronic condition, specifically cancer, a subpopulation of MDSCs has been observed, characterized by presenting major histocompatibility complex class II (MHC-II). In our laboratory, we utilized an acute model of SLE, MRL/MpJ-Fas^{lpr}/J, and observed the presence of MDSCs at two different times, defined as early (10-12 weeks) and late (14-16 weeks). Additionally, we identify a subpopulation of MDSCs expressing MHC-II in secondary lymphoid organs (spleen and axillary lymph nodes) and lungs. Furthermore, we determined that G-MDSCs expressing MHC-II correlate positively with IFN-γ producing CD4+ T cells whose effect is mainly seen in the lung. These results suggest that this subpopulation of MDSCs may be performing a novel mechanism that can modulate CD4+ T cells, contributing to the SLE pathogenesis.

35. 067. **SMALL EXTRACELLULAR VESICLES FROM METABOLICALLY REPROGRAMMED MESENCHYMAL STEM/STROMAL CELL AS A POTENTIAL IMMUNOSUPPRESSIVE MECHANISM FOR INFLAMMATORY AND AUTOIMMUNE DISEASES**

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Introduction: Inflammatory and autoimmune diseases affect the health and life of patients; their treatment has always been a clinical challenge. MSC can be used due to their immunoregulatory effects, making them an attractive strategy. Their activity is mediated mainly by paracrine factors Small extracellular vesicles (sEVs) released by MSCs are the principal mechanism through which they exert their biological effects. Our studies on human umbilical cord MSCs showed that metabolic reprogramming to glycolysis significantly improves their immunoregulatory capacity over proinflammatory T cells through inducing T regulatory cells. Therefore, in the present study we evaluated the role of sEVs from glycolytic and non-glycolytic UC-MSCs on their immunosuppressive properties *in vitro* and *in vivo* in a murine model of collagen-induced arthritis (CIA). **Materials and Methods:** We isolated sEVs from glycolytic and non-glycolytic UC-MSCs, characterizing them using nanoparticle tracking analysis (NTA) and flow cytometry (FACS). We assessed their immunosuppressive effects on peripheral blood mononuclear cells (PBMCs), proinflammatory T cells, and the induction of Tregs. The internalization of sEVs by T cells was analyzed via FACS and confocal microscopy. We also evaluated the effects on memory T-CD4 cells, including the phenotype of proinflammatory and anti-inflammatory cells and IL-10 production. The immunosuppressive activity of sEVs was further tested in vivo in a CIA mouse model. **Results:** Glycolytic MSC-derived sEVs significantly reduced T cell proliferation, decreased Th1 cells, and increased Treg cells in vitro. They were also internalized by memory T-CD4+ cells, reducing Th1 and Th17 cell percentages and increasing IL-10 production without affecting Tregs. In vivo, glycolytic sEVs reduced both the incidence and progression of CIA, correlating with decreased Th1 and Th17 cells in lymph nodes and peripheral blood. **Conclusion:** Glycolytic MSC-derived sEVs effectively modulate activated T cells, regulating their proliferation and phenotype. These sEVs demonstrated enhanced immunoregulatory capabilities and therapeutic potential for treating inflammatory and autoimmune diseases.

36. 130. **MULTIRREFRACTORY IMMUNE THROMBOCYTOPENIA ASSOCIATED TO A NOVEL CASP-10 VARIANT. NOT ALWAYS MEANING ALPS**

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OBJECTIVE: We present a 29-year-old male patient with a history of diagnosis of Evans Syndrome in 2012, treated by a pediatric hematologist with corticosteroids and rituximab. CASE REPORT: Between April 2014 and June 2015, Clinical Immunology followed him up, for screening of inborn errors of immunity (IEI); laboratory showed a CD4+ lymphopenia with 1.66% of double negative alpha-beta T cells, with high CD3+DR+, and decrease in pre and post-switch memory B-lymphocytes. He had no lymphoproliferation, only mild splenomegaly. Functional apoptosis tests could not be performed. The patient discontinued follow-up. In his transition to adulthood was followed by the Hematology Department, treated with corticosteroids, rituximab, mycophenolate mophetil and eltrombopag, with multiple refractoriness, splenectomized in November 2023. He restarted follow-up by Clinical Immunology, and gene sequencing for IEI was made in January 2024. The patient suffered a cervical demyelinating syndrome, with oligoclonal bands in cerebrospinal fluid in February 2024, treated with pulsed corticosteroids, intravenous immunoglobulin, and mycophenolate mophetil. Genetic testing was retrieved in June 2024, finding a heterozygous variant of uncertain significance for the caspase-10 gene (CASP10) in exon 6/6 (Chr2(GRCh38): c.738C>A p.Asp246Glu.), related to autoimmune lymphoproliferative syndrome type II (ALPS II). The patient's clinical manifestations: immune thrombocytopenia, demyelinating disease and the multirefractory nature of the treatments installed, are characteristics of CASP-10 dysfunction, but not typical of ALPS. Parental genetic study is pending completion. Therapy with sirolimus was started. CONCLUSION: This patient did not fulfill ALPS criteria, and only immune deregulation features were remarkable. Several authors highlight the importance of considering patients with CASP10 variants and immune deregulation as having a distinctive phenotype. Due to this

37. 192. **IMPORTANCE OF ERK5 IN THE DIFFERENTIATION AND FUNCTION OF T REGULATORY CELLS**

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Background: FoxP3+ regulatory T cells (Tregs) are critical for immune system homeostasis. TGF-B is essential for FoxP3 expression and crucial for Treg differentiation, maintenance, and function. ERK5, an atypical MAPK family member, plays a role in regulating transcription factors. Although TGF-B activates ERK5 in various cells, its role in Treg differentiation and function is unexplored. **Aim:** This study investigates ERK5's role in Treg biology. **Methods:** Naïve CD4+ T cells from C57BL/6, $ERK5^{f/f}$, $CD4^{cre}ERK5^{f/f}$, and $ERK5^{f/f}FoxP3^{cre}$ and $FoxP3^{cre}$ mice were cultured under Treg-polarizing conditions. The role of ERK5 in autoimmune diseases was studied using an experimental autoimmune encephalomyelitis (EAE) model induced by MOG^{35-55} peptide. **Results**: We found that phosphorylation of ERK5 is increased in Tregs cells when compared with the control group. Then, we show that pharmacological inhibition or genetic deficiency of ERK5 in CD4 T cells decreased the differentiation of Treg cells. Furthermore, $CD4^{cre}ERK5^{f/f}$ and $FoxP3^{cre}ERK5^{f/f}$ mice developed more severe EAE than $ERK5^{f/f}$ and control $FoxP3^{cre}$ mice, characterized by a reduction of Treg cells in draining lymph nodes. Furthermore, FoxP3+ cells isolated from the $FoxP3^{cre}ERK5^{f/f}$ animal were shown to possess less stability and lower suppressive potential when compared to FoxP3+ cells from the FoxP3Cre. RNA-seq analysis of ERK5-deficient Tregs indicated downregulation of ATG13, an essential component of the autophagy pathway, suggesting a potential link between ERK5 and autophagy in Treg cells. **Conclusion:** ERK5 is crucial for Treg differentiation, function, and stability. ERK5 deficiency worsens EAE, highlighting its role in immune balance. ERK5's regulation of autophagy in Tregs suggests a mechanistic link. Targeting ERK5 could be a therapeutic strategy for autoimmune diseases. **Footnote:** Supported by FAPESP, CRID, CNPq, and CAPES.

38. 276. **AGE-ASSOCIATED B CELLS AND PROINFLAMMATORY CYTOKINES IN SYSTEMIC LUPUS ERYTHEMATOSUS: A PRELIMINARY CASE-CONTROL STUDY**

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Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of autoantibodies and an overactive immune system, leading to inflammation and organ damage. Among the different B cell subtypes involved in SLE, age-associated B cells (ABCs) have garnered particular interest. These atypical B cells, identified by the expression of CD11c and T-bet, are found in higher levels in individuals with autoimmune diseases, chronic infections, and older age. ABCs are associated with increased production of autoantibodies and proinflammatory cytokines, suggesting a significant role in SLE pathogenesis. **Objective:** To compare B and T cell populations, including ABCs, and measure serum cytokine levels in patients with SLE and healthy controls to identify key immunological differences. **Method:** Peripheral blood samples were collected from all participants. After removing erythrocytes using a lysis buffer, B and T cell subpopulations were identified through flow cytometry. Serum cytokine levels were measured using the LEGENDplex™ assay. **Results:** Preliminary analysis of nine SLE patients and five healthy controls showed no notable differences in T lymphocyte populations. However, a significant increase in B lymphocytes (CD20+) was observed in SLE patients ($p<0.02$), along with a trend towards higher absolute B cell counts ($p < 0.06$). In terms of subpopulations, memory B cells (CD20+ CD24+ CD38-) were slightly reduced ($p < 0.07$). Importantly, ABCs (CD20+ CD11c+ T-bet+) were significantly elevated in both proportion and absolute numbers in SLE patients ($p<0.03$). Patients with higher ABC counts also presented with more severe clinical scores, as measured by SLEDAI. Serum cytokine analysis revealed significantly elevated levels of IL-8 and IP-10 ($p < 0.05$), with a trend towards increased IL-1β and IFN- γ levels ($p < 0.06$) in SLE patients. **Conclusion**: SLE patients show marked changes in B cell populations, particularly a significant increase in ABCs, underscoring their potential role in disease development.

39. 329. **THE CATION CHANNEL TMEM176B IS A DRUGGABLE PROTECTIVE FACTOR IN AUTOIMMUNE DISEASE**

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Background: Autoimmunediseasesarisewhentheimmunesystemlackstolerancetoself-antigens, causing inflammation and tissue damage. Fostering immunosuppression while preservinghostprotectivefunctionischallenging. Hence,understandingthemolecularprocessesunderlyingpathologyisessentialfordevelopingeffectivetherapies. TMEM176B, an immunoregulatory cation channel, has been associated with protection inautoimmune settings. Moreover, we reported its roleas an inflammasome inhibitorcapableof modulating the fate of CD8 T cells in cancer and viral infections. We hypothesized thatTMEM176BcouldalsocontrolautoimmunediseasesbymodulatinginflammasomeactivationandCD8 Tcells. **Methodology:** The experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosiswas used. Experimental approaches included microscopy, flow cytometry, fluorometry

andELISA.Bioinformaticanalysiswasconductedon SystemicLupusErythematosus(SLE)patientdata. **Results:** *Tmem176b*$^{-/-}$mice showed more severeEAE, increased microgliosis and demyelinationversusWTand*Tmem176b-/-Casp1*-/-mice,suggestingaroleforinflammasomesinaggravating neuroinflammatory disease. Additionally, Tmem176b expression decreased inmicroglia of EAE mice versus healthy controls. Flow cytometry revealed an increase in CD8progenitor exhausted T cells in the spinal cord of *Tmem176b*$^{-/-}$versus WT and *Tmem176b*$^{-/-}$*Casp1*$^{-/-}$mice. A significantly positive correlation was observed between EAE severity and Texhaustedtransitorycellsin*Tmem176b*$^{-/-}$mice,suggestingapathogenicroleofthisexhaustedTcell sub-population, whichretainscytotoxicityfunction. Inhumans,weidentifiedTMEM176BSingleNucleotidePolymorphism(SNP)rs2072443asa loss-of-function mutant associated with increased clinical manifestations in SLE patients,includingfever,leukopenia,and hemolysis. Furthermore, TMEM176B could be pharmacologically modulated by the immunoregulatorylectin Galectin-1. Galectin-1 interacted with TMEM176B to inhibit inflammasome activity,activated its ion-transportactivity,and restored its functionin the SNPvariant.Finally,Galectin-1controlledEAE in aTmem176b-dependentmanner. **Conclusions:** TMEM176B emerges as a potential druggable target that controls autoimmune diseases,throughan inflammasome-CD8 Tcell exhaustionpathway.

40. 342. **MODULATORY EFFECT OF SLAN+ MONOCYTES ON OTHER MONOCYTE AND LYMPHOCYTE POPULATIONS EXPOSED TO INFLAMMATORY STIMULATIONS**

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Background Traditionally, three monocyte subsets are defined based on CD14 and CD16 expressions. Among the nonclassical monocytes, a Slan+ subgroup (slanMo) is reported to elicit the highest inflammatory responses. SlanMo express a carbohydrate modification of P-selectin glycoprotein ligand 1 (PSGL-1) attached to a structured sugar called 6-sulfo- LacNAc and has been shown that these monocytes can modulate other cell populations depending on the stimulus and their proportion. **Aim** We aimed to analyze the effect of SlanMo on the response to apoptotic-bodies (AB), LPS, and CD3-T-lymphocyte proliferation using in vitro clearance and reconstitution assays. **Methodology** We utilized highly purified Slan+ and Slan- monocyte populations obtained through cell sorting and then mixed them in fixed proportions of Slan+:Slan- (10:90 to 60:40). Cultures were stimulated with LPS and AB from K562 cells, and proliferation was measured using CFSE. Fluorescence microscopy images were captured with an EVOS- M5000. **Results** We observed a differential response between stimuli, which depended on the increasing proportion of SlanMo in the reconstitution and proliferation assays. In the LPS assays, the SlanMo fraction showed an increase in the median fluorescence of surface markers related to activation and maturation. In contrast, with AB, the SlanMo fraction decreased in maturation-related surface markers compared to unstimulated cells. Additionally, some maturation markers increased in the Slan- fraction, with a rise in median fluorescence when stimulated with AB. Unstimulated cultures showed increased cytokine levels, while LPS stimulation decreased IL-10 levels; AB stimulation increased IL-10 levels and decreased IL-6 and IL-1 levels. **Conclusions** The ability of SlanMo to modulate responses to these stimuli and interact with other immune system components underscores their significant role in regulating immune responses. Our findings indicate that contrary to being solely associated with the inflammatory response, SlanMo responses are conditioned by the specific stimulus, further highlighting their high plasticity.

41. 378. **HIGHLIGHTS IN THE TRANSCRIPTOMIC PROFILE OF PLATELETS FROM PATIENTS WITH RHEUMATOID ARTHRITIS**

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Background. Rheumatoid arthritis (RA) is one of the most prevalent autoimmune diseases globally. Components of hematopoietic lineage, including platelets, play crucial roles in RA. These anucleate structures participate in hemostasis, inflammation, and tissue repair. In RA, platelets contribute to pathogenesis by releasing prothrombotic and inflammatory molecules. Patients with RA exhibit a peripheral blood transcriptomic profile associated with platelet function, but specific platelet transcripts in patients with RA remains unknown. Identifying these molecular candidates may enable the discovery of potential biomarkers for an early diagnosis of RA. **Objectives.** Analyze the gene expression profile of platelets in patients with RA, categorized by serological findings and disease activity, and compare it with healthy donors. **Methods.** This study enrolled 22 patients with RA along with 6 healthy donors. Platelets were isolated using differential centrifugation, and purity was estimated by flow cytometry. RNA extraction, quality assessment, library preparation, and sequencing were conducted using commercial kits. Quality control and fastq files were processed, and changes in transcriptomic signatures between patients with RA and healthy donors were evaluated using DESeq2. Differentially expressed genes were selected, and functional analysis was performed using Gene Ontology (GO) and gene network interaction. **Results.** Significant differences were observed in RA patients compared to healthy donors, highlighting genes related to extracellular matrix structure including FN1 and MMP9, and chemokine responses such us CCL2. In addition, relevant genes were associated with lipid responses, cytokine/chemokine signaling, and RAGE binding. Finally, key genes were identified as potential molecular biomarkers including ACVR2B, TNFAIP6, TRO, and IL40. **Conclusion.** The platelet transcriptomic profile in RA patients differ to healthy donor group, suggesting platelets and their products as key players in RA pathophysiology. Changes in the expression of platelet genes have the potential to serve as markers for early diagnosis, to follow disease development, progression, and disease activity.

42. 398. **CHARACTERIZATION OF TISSUE-RESIDENT MEMORY B CELLS IN THE KIDNEY OF A MOUSE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS**

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Systemic autoimmune diseases, such as Systemic lupus erythematosus (SLE), are typically characterized by the activation of self-reactive B cells, leading to the generation of self-reactive memory B cells (Bmem) and plasma cells. Consequently, SLE patients have increased numbers of circulating memory B cells, which often correlate with disease activity. Recent studies have demonstrated that, in the context of infection, Bmem is not only maintained in the circulation but also acquire tissue residency in affected organs such as the lungs. Whether tissue-resident Bmem can also arise in autoimmune disease and contribute locally to tissue damage has not yet been explored.
In this work, we used the BWF1 mouse model, where 7-month-old female mice spontaneously develop a lupus-like disease, including severe glomerulonephritis and accumulation of autoantibodies. By combining intravascular labeling and immune phenotyping to distinguish circulating vs non-circulating lymphoid cells, we show that BWF1 mice with glomerulonephritis exhibit a remarkable accumulation of non-circulating B cells in the kidneys. A significant fraction of these non-circulating kidney B cells is class-switched and IgM^+ memory B cells that express the memory markers CD73 and PD-L2. Notably, these class-switched memory B cells in the kidney also express the signature markers of tissue residency, CXCR3 and CD69. Additionally, the kidneys of lupus-prone mice harbor a significant proportion of self-reactive plasma cells. Remarkably, we demonstrated that these Bmem subsets are resistant to B cell-depletion therapy with Rituximab, in contrast to their circulating counterpart, highlighting a relevant limitation to the current use of Rituximab in SLE patients. In conclusion, our results show that in the kidney of mice with spontaneous lupus, there is an accumulation tissue-resident memory B cells that are resistant to elimination. This population, previously observed only in the lungs of infected mice, may play a role in the pathogenesis of this disease.

43. 423. **HIGH-DIMENSIONAL IMMUNOPHE-**

NOTYPING OF MYELIN-OLIGODENDROCYTE GLYCOPROTEIN ANTIBODY-ASSOCIATED DISEASE

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Myelin-oligodendrocyte glycoprotein (MOG) antibody-associated disease (MOGAD) is a rare autoimmune disorder characterized by autoantibodies against MOG in the central nervous system. Due to its recent description and rarity, the pathogenesis of MOGAD remains poorly understood, and specific therapies are lacking. We employed computational analysis of high- dimensional spectral flow cytometry data from two patient cohorts to identify immune dysregulations and disease signatures in MOGAD. Compared to healthy individuals, MOGAD patients exhibited significant alterations in peripheral immune profiles. We observed an expansion of BND2 ($p<0.0001$) and DN2 ($p=0.00033$) B cells, two subsets that are associated with the extrafollicular pathway. This suggests a potential activation of circulating autoreactive B cells and their differentiation into antibody-secreting cells outside of lymphoid follicles. Additionally, altered expression of Fcγ receptors CD16 and CD64 in natural killer (NK) cells and non-classical monocytes, respectively, highlights the potential role of dysregulated FcγR-mediated effector functions in MOGAD pathogenesis. The lower expression of CD16, coupled with signs of NK cell exhaustion, may help to explain the variable efficacy of Rituximab, a therapeutic antibody targeting CD20+ cells, in reducing MOGAD relapses. Furthermore, MOGAD patients had a lower abundance of CXCR3+ central memory CD4 T cells in circulation ($p<0.0001$), a subset that potentially migrates to sites of inflammation. This study provides a comprehensive immunophenotypic characterization of MOGAD, offering novel insights into its immune landscape and paving the way for future hypothesis-driven research to develop targeted treatment strategies.

44. 428. **TARGETED INHIBITION OF PRO-INFLAMMATORY SLAN+ MONOCYTES USING WGA-FUNCTIONALIZED NANOPARTICLES ENCAPSULATING ITACITINIB: A NOVEL THERAPEUTIC APPROACH**

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Background: 6-sulfoLacNAc+ (slan) monocytes (SlanMo) are a subset of non-classical monocytes known for their pro-inflammatory role in various diseases, including autoimmune disorders such as systemic lupus erythematosus (SLE). Previous research from our group has highlighted the reduction and dysfunction of circulating non-classical monocytes and their recruitment to inflammatory sites in patients with SLE. **Objective**: This study aimed to evaluate the therapeutic potential of itacitinib (ITA) encapsulated in wheat germ agglutinin (WGA)-functionalized F127-emulsified polylactic-co-glycolic acid (PLGA) nanoparticles (WGA/F127/PNPs) to selectively target and inhibit the JAK-STAT pathway in slan+ monocytes. **Methods:** We examined monocyte subpopulations in SLE patients (n=50, from ARTMEDICA IPS) and healthy controls (n=37) using flow cytometry, focusing on the surface expression of CD14, CD16, HLA-DR, and Slan. Curcumin- and ITA-loaded WGA/F127/PNPs were prepared. The binding and internalization of curcumin-WGA/F127/PNPs by leukocytes and monocyte subpopulations from healthy controls were assessed using flow cytometry. The effect of ITA-WGA/F127/PNPs versus free ITA on IFN-γ-stimulated monocyte subsets was evaluated by analyzing the expression of HLA-DR, CD86, CD69, and pSTAT1, as well as cytokine synthesis. **Results:** SLE patients showed a notable reduction in circulating SlanMo compared to healthy controls, with statistical significance ($p<0.001$). This decrease might be due to their recruitment to inflamed sites, highlighting the body's effort to combat inflammation. The SlanMo demonstrated remarkable efficiency in internalizing WGA/F127/PNPs, unlike their slan-monocyte counterparts. Excitingly, ITA-WGA/F127/PNPs successfully reduced

surface expression of HLA-DR, CD69, and CD86, as well as STAT1 phosphorylation and cytokine production in IFN-γ-stimulated slan+ monocytes. **Conclusion:** The study demonstrates that encapsulating itacitinib (ITA) in WGA/F127/PNPs nanoparticles effectively targets and inhibits the JAK-STAT pathway in 6-sulfoLacNAc+ (slan) monocytes. These findings offer promising insights into therapeutic strategies to modulate immune responses in SLE patients.

45. 442. **HUMAN KERATINOCYTES DELIVER TOLEROGENIC SIGNALS TO LANGERHANS CELLS THROUGH A GALECTIN-7-DEPENDENT PATHWAY INVOLVING IL-10 AND REGULATORY T CELL EXPANSION**

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Background: The skin barrier involves a dynamic interplay between keratinocytes (KCs), Langerhans cells (LCs) and T cells, which preserves homeostasis. Disruption of this equilibrium triggers chronic inflammatory skin diseases such as psoriasis. Previously, we demonstrated in transgenic mouse models, that Galectin-7 (GAL7) fuels immunoregulatory programs in the skin of psoriatic mice. However, the clinical relevance of these findings in human settings and the molecular pathways underpinning these regulatory circuits remain uncertain. **Objective:** To investigate the role of GAL7 in fostering tolerogenic programs between human KCs and LCs. **Methods:** Publicly available gene expression datasets (GSE 133355, GSE14905) of psoriatic patients were processed using bioinformatic tools. Human monocyte-derived LC-like cells were differentiated from $CD14^+$ using GM-CSF, IL-4 and TGF-beta with or without recombinant GAL7 or with conditioned medium (CM) of HaCaT KCs. IL-10 was assessed by ELISA; $CD4^+$ cell proliferation and $CD4^+CD25^{high}FoxP3^+$ Tregs were determined by flow cytometry. **Results:** Transcriptomic analysis of psoriasis patients skin revealed higher *LGALS7* (encoding GAL7) in lesioned and non-lesioned skin compared to healthy individuals and a considerable reduction in GAL7 expression in patients treated with etanercept (anti-TNF-alpha) and brodalumab (anti-IL-17R) versus untreated patients ($p<0.01$ and $p<0.05$). We found that LCs differentiated from human monocytes did not produce IL-10. However, exposure to GAL7 at the beginning of the differentiation process induced IL-10 release and inhibited proliferation of $CD4^+$ T cells along with an increase in frequency of FoxP3+ Tregs within LC-T cell co-cultures. These effects were recapitulated when LCs were differentiated in presence of CM of imiquimod (TLR7 agonist) or TGF-beta1-stimulated HaCaT, and were suppressed by an anti-GAL-7 antibody ($p < 0.01$), suggesting a paracrine immunoregulatory role of this lectin. **Conclusion:** Our findings reveal a critical role for GAL7 in mediating human KCs-LCs crosstalk, promoting immune tolerance in the skin and offering a promising avenue for developing novel therapies for psoriasis.

46. 467. **FOSTER STAGES ACCORDING TO THE DELAY IN DIAGNOSIS OF OCULAR CICATRICIAL PEMPHIGOID**

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Background: Ocular cicatricial pemphigoid (OCP) is an autoimmune disease that can end in blindness. Although it is known that it progresses, scarce data was published about the extent of this progression according to the different stages of the disease and the times that could be relevant for this to occur. **Objective**: To describe the delay times in diagnosing OCP and the Foster stage at diagnosis. Factors associated with

worse initial stages of the disease were evaluated. **Methods**: A retrospective, observational, and multicenter study was performed. The medical records of patients diagnosed with OCP from different rheumatology healthcare centers in Argentina were reviewed from May 2006 to January 2024. The initial Foster stage was recorded at the first visit, and the patients were interrogated about the time they had symptoms related to OCP until the date of diagnosis of the disease. A logistic regression analysis was performed to identify the variables associated with severe Foster stages (2, 3, and 4). **Results**: A total of 168 patients were included. Females were the 73.2%, with a median age at diagnosis of 64.1 (SD 13.2) years. The median diagnostic delay was 36 (IQR 107) months. Seven (3.5%) patients had Foster stage 0 at diagnosis. Patients with Foster stages 1, 2, 3, and 4 had a median delay of 23 (IQR 83.5), 44.5 (IQR 92), 62.5 (IQR 72), and 56 (IQR 1295) months, respectively. Logistic regression analysis showed that a 30-month delay in diagnosis was associated with greater severity in Foster stages (OR 4.1; 95% CI 1.9 to 8.9; p <0.001) adjusted for hypergammaglobulinemia and occupational environmental exposure. **Conclusion**: In our series, we observed delay times at the start of treatment with worse Foster stages. Scars were observed with a delay of 23 months. Severe stages seem to manifest after 30 months of disease.

47. 481. **SPECIFIC PROTEIN CARGO IN EXTRACELLULAR VESICLES FROM PATIENTS WITH RHEUMATOID ARTHRITIS**

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Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic joint inflammation. Early intervention in these patients is pursued to improve clinical outcomes. Detection of autoantibodies divides patients into seronegative (SN) and seropositive (SP) groups, with the latter having a worse prognosis and phenotypical changes in extracellular vesicles (EVs, membrane-bound particles) compared to SN patients. EVs from RA patients are considered important sources of autoantigens and alarmins, contributing to the production of cytokines critical to RA pathology. Specific protein cargos in circulating EVs may contribute to systemic inflammation in RA patients and serve as diagnostic biomarkers. Blood was collected from healthy donors (HD) and RA patients, classified by seropositivity and disease activity (active or remission according to the DAS28 clinical score). EVs were isolated by differential centrifugation and filtration, then characterized fresh by flow cytometry. Proteomic analysis was performed using liquid chromatography coupled with tandem mass spectrometry. Partial least squares discriminant analysis (PLS-DA) and fold change (FC) analysis were implemented to identify the proteins that best discriminated the study groups. The EV protein content of RA patients, compared to HD, showed an upregulation of the complement component CFHR3, which was associated with DAS28. EVs from SP patients exhibited higher levels of aggrecan (FC = 2.41) and PIGR protein (FC = 1.43) compared to HD. Aggrecan correlated with rheumatoid factor levels in serum (r = 0.7), and PIGR with IL-6 cytokine (r = 0.61). SN patients showed proteins related to cholesterol transport. CRP in EVs distinguished active patients from those in remission, who particularly expressed MSI2. This analysis identified characteristic EV proteins in RA patients that could be potential biomarkers for early and pre-clinical diagnosis.

48. 506. **CONDITIONS ASSOCIATED WITH POLYCLONAL HYPERGAMMAGLOBULINAEMIA IN A TERTIARY HOSPITAL**

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Background: Polyclonal hypergammaglobulinaemia (PHG) is the overproduction of immunoglobulins by plasma cells of various clones. It's associated with a variety of disorders: hepatic, autoimmune, inflammatory infectious and neoplastic. Objectives: To determine the causes of PHG in 1 tertiary care hospital in Mendoza. To assess whether CRP level is associated with any cause of PHG. Methods: Protein electrophoresis (PE) performed from 01/07/2021 to 30/06/2023 using CAPILLARYS PROTEIN(E)6 was retrospectively reviewed. Patients (p) with gammaglobulin ≥2 g/dl were selected. Inclusion criteria: presence of PHG and certain final diagnoses. The value of CRP was evaluated as an indicative tool for the etiology of PHG. A protocol was completed with demographic, clinical, and laboratory data. Descriptive statistics, univariate analysis, and ROC curves were performed. Results: 6117 PE were analysed, 337p presented PHG (2.03-6.44 g/dl), 263 met inclusion criteria. Associated etiologies were: systemic autoimmune diseases (SAID) 106p (40.3%), liver diseases (LD) 92p (34.98%), infectious diseases 49p (18.63%), hematological diseases 28p (10.65%), nonhematological neoplastic diseases 16p (6.58%). The most prevalent SAID were SLE 39p (36.76%), Sjögren's syndrome 29p (27.36%), RA 21p (19.81%), vasculitis 4p (3.77%). When comparing etiologies according to gamma level, in the PHG 2-2.99gr/dL group (n= 210) the main etiology was SAID 87p (41.43%), while in the PHG ≥3 gr/dL group (n=53) the most prevalent was LD 26p (49.06%). In the SAID group, the median CRP was lower than the rest 6.06mg (2.3-13.8) vs 16.05mg/dL (6.03-46.09), p 0.00001. The AUC of ROC curve for CRP ≤10.5mg/dL was 0.68 (95%CI 0.6-0.74); sensitivity 71.43% (95%CI 60.5-80.8), specificity 65.05% (95%CI 55-74.2), LHR+ 2.04 and LHR-0.44 for SAID as a cause of PHG. Conclusion: In our sample, SAID was the most prevalent cause of PHG, but LD was more frequent when PHG was ≥3gr/dl. Patients with PHG and CRP ≤10.5 were associated with SAID.

49. 592. **THE EFFECTS OF HUMAN CHORIONIC GONADOTROPIN TREATMENT IN AN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MOUSE MODEL OF INDUCED MULTIPLE SCLEROSIS**

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Multiple Sclerosis (MS) is a neurodegenerative disease of the Central Nervous Systems. The most common clinical presentation is relapsing-remitting MS. Pregnancy has a significant impact on MS, typically reducing the frequency of relapses. The pregnancy associated hormone, human chorionic gonadotropin (hCG), has immunosuppressive functions. The commercial forms of hCG are commonly used in artificial reproductive techniques (ART). However in the context of MS, many studies have reported increased relapses after ART using this hormone. The aim of our study was to evaluate the effects of urinary (u) and recombinant (r) hCG on clinical symptoms in a mouse model of induced MS. Experimental Autoimmune encephalomyelitis (EAE) was induced in female C57BL/6 mice using the MOG35-55 peptide dissolved with Complete Freund's adjuvant and a dose of Pertussis Toxin (PT). Two days later, a second dose of PT was administered. hCG treatment began with a loading dose of uhCG (20IU) or rhCG (1ug) administered on the first day of immunization to achieve the required dose of hCG. Doses chosen for this experiment were similar to those expected during the first trimester of pregnancy. From day 1 of EAE induction, mice were injected with 10IU uhCG or 0.5µg of rhCG every second day for 16 days. Disease course was evaluated by a clinical score. Statistical analysis was performed using t-test or Two-way ANOVA. Data were shown as mean±SD.
During EAE induction, hCG treatment significantly accelerated the onset of the disease (day; uhCG:11.0±1.4**,rhCG:11.5±1.1*,PBS:12.8±0.5,**p<0.028vsPBS,*p<0.041vsPBS) and increased the clinical scores during relapse phase on days 11, 12 and 13 (day11; uhCG:1.0±1.2*,rhCG:1.0±1.2*,PBS:0.0±0.0; day12; uhCG:1.8±1.5**,rhCG:1.9±1.5**,PBS:0.1±0.2; day13; uhCG:2.5±1.2**,rhCG:2.5±1.1**,PBS:1.3±0.8,*p<0.05vsPBS,**p<0.028vsPBS)
In summary, our work revealed that treatment with hCG worsens the clinical course of the disease, consistent with studies conducted in humans. Further research is needed to elucidate the mechanisms underlying these results.

50. 647. **EVALUATING INOS INHIBITION EFFECTS IN AN EXPERIMENTAL MODEL OF TYPE 1 DIABETES**

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Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the destruction of pancreatic beta cells by immune system T cells. Currently, insulin therapy remains the only available treatment, and there is a significant need for effective alternative therapies. Anti-CD3 drugs are an approved therapeutic strategy to halt beta cell destruction, although their efficacy is associated with adverse effects. The inducible nitric oxide synthase (iNOS) enzyme plays a key role in beta cell loss through its interactions with immune cells. This study aimed to examine the effects of an iNOS inhibitor (iNOSX) on the onset and progression of insulitis by evaluating the immune response in non-obese diabetic (NOD/ShiLtJ, NOD) mice. Pre-insulitis female NOD mice (PRE) were treated with iNOSX or vehicle control (Co, n=6-7/group) for 2 weeks, followed by a 2- week monitoring period. Pre-diabetic female NOD mice (DIS) were treated with iNOSX or Co (n=10/group) for 4-weeks, followed by 4-weeks of monitoring. The onset and progression of the disease were assessed by monitoring blood glucose, plasma insulin, body weight, and glucose tolerance at the end of the treatment. At the end of the study, the mice were sacrificed, and pancreatic tissue (PANC) samples were collected for T cell analysis by flow cytometry. In the PRE iNOSX, a significant decrease ($p<0.05$) in fasting glucose levels was observed vs. Co. iNOSX treatment also reduced the frequencies of Th1 and the Th1/Th2 ratio in PANC in PRE vs Co ($p<0.05$). At the end of the study, DIS iNOSX had higher plasma insulin levels than the Co group ($p<0.05$). Additionally, there was a trend towards delaying the onset of T1D in the DIS group treated with iNOSX vs. Co. These findings suggest that iNOSX treatment affects immune populations at the pancreatic level, potentially delaying type 1 diabetes progression and preserving beta cell function.

51. 681. ANALYSIS OF THE ASSOCIATION BETWEEN NLRP3 GENETIC VARIANTS (rs10157379 AND rs10744558) AND SUSCEPTIBILITY TO RHEUMATOID ARTHRITIS

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Background: Rheumatoid Arthritis (RA) is a chronic autoimmune disease characterized by inflammation and joint damage, with a strong genetic component influencing the susceptibility. The NLRP3 inflammasome is involved in the immune response, and its genetic variants have been linked to various inflammatory conditions. However, the role of specific *NLRP3* variants in RA susceptibility remains unclear. **Objectives:** To investigate the association between *NLRP3* rs10744558 C>G and rs10157379 C>T variants and the susceptibility to RA in a Brazilian population. **Methods**: This case-control study included 262 RA patients and 168 healthy controls. RA diagnosis was based on the American College of Rheumatology criteria, and disease activity was assessed using the DAS28 score. Anti-cyclic citrullinated peptide (anti-CCP) and rheumatoid factor (RF) were measured. Genomic DNA was extracted from peripheral blood mononuclear cells, and genotyping of the *NLRP3* variants (rs10744558 C>G and rs10157379 C>T) was performed using real-time polymerase chain reaction. Statistical analysis was performed using chi-square tests

for genotype frequencies and logistic regression models to assess the association. **Results:** The genotype distributions of rs10744558 (CC, CG, GG) and rs10157379 (CC, CT, TT) did not differ significantly between RA patients and controls in allele-based or genotype-based models. No association was found in the dominant, recessive, or overdominant models for either variant. The CC, CG, GG, and CC, CT, TT genotypes of the two *NLRP3* variants (rs10744558 C>G and rs10157379 C>T, respectively) were not associated with anti-CCP and RF ($p > 0.05$) in the dominant, recessive, and overdominant models (all $p > 0.05$). **Conclusion:** The study showed no association between the *NLRP3* rs10744558 and rs10157379 variants with susceptibility to RA, as well as with autoantibodies biomarkers of RA. Further research is needed to explore the role of other genetic and environmental factors in the RA pathogenesis.

52. 695. EVALUATION OF INTERLEUKIN 1 BETA GENE VARIANTS IN PSORIASIS SUSCEPTIBILITY

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Background: Psoriasis (PsO) is an immune-mediated disease that affects the skin. Its pathophysiology is complex, involving both cutaneous tissue and immune cells. Although its etiology remains unclear, evidence suggests that genetic, epigenetic, and environmental factors can influence the development of the disease. Interleukin-1β (IL-1β) is a key mediator in the immune response of PsO, promoting the differentiation of T helper 17 cells and the production of other pro-inflammatory cytokines, which perpetuates the inflammatory cycle of the disease. **Objective**: This study aimed to evaluate the *IL1B* A>G (rs16944) and *IL1B* G>A (rs1143634) gene variants as predictors of PsO susceptibility. A case-control study was conducted, enrolling 100 patients with PsO and 100 healthy controls from the Psoriasis Outpatient Clinic at the University Hospital of Londrina, Brazil. The *IL1B* rs16944 and rs1143634 variants were analyzed using real-time polymerase chain reaction (PCR). **Results**: A significant association was found between the *IL1B* variants and PsO. For the *IL1B* G>A (rs1143634) variant, the AA genotype demonstrated the strongest negative association with PsO [odds ratio (OR) = 0.07, 95% confidence interval (CI) = 0.01-0.38, $p = 0.002$], indicating a protective effect. In contrast, the *IL1B* A>G (rs16944) variant showed a positive association with PsO, with the AA genotype having the highest association (OR = 5.11, 95% CI = 1.91-13.65, $p = 0.001$), indicating a 5.11-fold increased likelihood of being associated with PsO. The sample size and the matching of sex and age between the groups provided robust statistics, although the study design does not permit causal inferences. **Conclusions**: These findings suggest that the *IL1B* G>A (rs1143634) variant may confer a protective effect against PsO development, while the *IL1B* A>G (rs16944) variant may contribute to increased susceptibility to the disease.

53. 698. ASSOCIATION OF NLRP3 GENE VARIANTS WITH SUSCEPTIBILITY TO ANKYLOSING SPONDYLITIS, HLA-B27 STATUS, AND DISEASE ACTIVITY

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Introduction: Spondyloarthritis (SpA) pathogenesis is multifactorial, involving genetic, environmental, and immunological factors. Genetic components, particularly inflammasomes such as the *NLRP3* gene, have been implicated in activating inflammatory pathways.
Objective: To assess whether the *NLRP3* C>G (rs10754558) and *NLRP3* T>C (rs10157379) variants are linked to SpA susceptibility, HLA-B27 status, and disease activity. **Methods:** A total of 207 healthy controls and 103 AS patients aged 18 to 70 were recruited from the University Hospital of Londrina. Demographic and clinical data, including HLA-B27 status, high-sensitivity C-reactive protein (hsCRP) levels, and erythrocyte sedimentation rate (ESR), were collected. Genotyping of the *NLRP3* variants was performed using real-time polymerase chain reaction (PCR). Logistic regression models and chi-square tests were used for statistical analyses. **Results:** The *NLRP3* C>G (rs10754558) variant was significantly associated with AS in the recessive model. Individuals with the GG genotype showed a lower likelihood of AS compared to CC or CG genotypes (OR = 0.419, p = 0.048). The CG genotype was also associated with increased HLA-B27 positivity compared to CC genotype carriers (OR = 2.554, p = 0.036). However, no significant association was found between the *NLRP3* T>C (rs10157379) variant either with AS susceptibility or with HLA-B27 status. Regarding disease activity, no significant associations were found between both *NLRP3* variants and BASDAI scores. **Conclusion:** The *NLRP3* C>G (rs10754558) variant, particularly the GG genotype, may confer protection against AS and is linked to HLA-B27 positivity. However, these variants were not associated with disease activity as measured by BASDAI. Further research is warranted to elucidate the functional implications of these genetic differences in AS pathogenesis.

54. 701. DOUBLE POSITIVITY OF ANTI-SMOOTH MUSCLE AND ANTI-LIVER KIDNEY MICROSOMAL ANTIBODIES AS A FINDING IN SUSPECTED AUTOIMMUNE HEPATITIS

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Background: Autoimmune hepatitis (AIH) is a chronic inflammatory disease that, if left untreated and depending on its progression rate, can be life-threatening and may require organ transplantation for survival. An atypical case from a biochemical standpoint is described, where a pediatric patient with multiple autoimmune pathologies presents with concomitant patterns corresponding to both type-1 and type-2 autoimmune hepatitis. **Objectives:** To describe the case of overlapping patterns compatible with anti-smooth muscle antibodies (ASMA actin) and anti-liver kidney microsomal antibodies (LKM). **Methods:** Indirect immunofluorescence was performed on rat liver, kidney, and stomach sections (Biocientífica©). **Results:** A 3-year-old patient with a history of type 1 diabetes, autoimmune thyroiditis, and celiac disease was admitted to the emergency room for vomiting and fever. She presented with altered liver function tests and slight hypergammaglobulinemia. Due to the gradual increase in transaminases during hospitalization, the patient was evaluated for suspected hepatopathy, obtaining positive results for ASMA actin and LKM, the first corresponding to a diagnostic marker of type-1 AIH and the second to type-2 AIH. Likewise, a result of antinuclear antibodies was obtained, with a nucleolar pattern 1/80, homogeneous 1/160 with a linear fibrillar cytoplasmic pattern (the latter compatible with anti-actin antibodies). **Conclusion:** The patient presented repeated positive results for both patterns, which is not reported in the literature and is a striking finding since both patterns have high specificity for different types of autoimmune hepatitis. This result is of biochemical interest due to its infrequency and highlights the importance of multidisciplinary work, as the multiple presence of autoantibodies that explain her various pathologies may refer to an innate error of immunity and explain in some way the overlap of immunofluorescence patterns.

55. 706. EVALUATION OF IL18 GENETIC VARIANTS IN ANKYLOSING SPONDYLITIS: ASSOCIATION WITH HLA-B27 AND DISEASE ACTIVITY

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Introduction: Spondyloarthritis (SpA) comprises a group of chronic inflammatory diseases, including ankylosing spondylitis (AS), which primarily affects the axial skeleton. The pathogenesis of AS involves genetic and immunological factors, with emphasis on the inflammasome pathway, which activates pro-inflammatory cytokines such as interleukin-18 (IL-18). Genetic variants in the *IL18* may influence IL-18 expression and have been studied for their potential associations with various diseases, particularly those related to the immune system. **Objective**: The aim of this study was to evaluate the association between the *IL18* -137 G>A (rs187238), *IL18* -105 G>A (rs360717), and *IL18* +105 T>G (rs549908) genetic variants with AS susceptibility, HLA-B27 positivity, and disease activity. **Subjects and Methods**: This case-control study included 207 healthy controls and 103 patients with AS recruited from the University Hospital of Londrina. Disease activity was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Demographic, clinical, and HLA-B27 status information were collected. Genomic DNA was extracted from peripheral blood mononuclear cells and the *IL18* variants were genotyped using real-time polymerase chain reaction. Logistic regression models were used to evaluate associations between the *IL18* genetic variants, AS susceptibility, disease activity, and HLA-B27 status. **Results**: The *IL18* rs187238, rs360717, and rs549908 variants showed no significant association with AS susceptibility (p>0.05). HLA-B27 positivity was more frequent in AS patients but it was not associated with these *IL18* variants. Disease activity (BASDAI≥4) showed no association with the *IL18* variants (p>0.05). Although patients with the *IL18* rs187238 CC genotype and those with the *IL18* rs549908 TT genotype showed slightly higher BASDAI scores than those carrying other genotypes, the difference was not significant. **Conclusion**: This study found no significant association between the *IL18* rs187238, rs360717, and rs549908 variants with AS susceptibility, HLA-B27 status, or disease activity. Further research is needed to clarify the role of *IL18* in AS pathogenesis.

56. 707. ERYTHROCYTE SENESCENCE IN AUTOIMMUNE HEPATITIS

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Autoimmune hepatitis (AIH) is a chronic liver disease of unknown cause characterized by circulating autoantibodies, increased serum IgG concentrations, and aminotransferase levels. The aetiology involves a breakdown in the immunotolerance against hepatocytes triggered by environmental factors in genetically predisposed individuals, possibly through molecular mimicry. In this pathology, hepatic iron accumulation is linked to the progression of liver damage. We propose that there may be a connection between enhanced red blood cell (RBC) senescence, subsequent erythrophagocytosis, and the elevated hepatic iron levels observed in these patients. Therefore, our study aims to evaluate markers of erythrocyte senescence, such as CD47, phosphatidylserine (PS), and membrane-bound IgG in senescent RBCs (SeRBCs), young RBCs (YRBCs), and total RBCs in AIH patients (n=20) undergoing immunosuppressive treatment, using flow cytometry. We observed a decrease in CD47 expression in the RBCs of AIH patients vs. the

control (Co) group (n=18) ($p < 0.001$). There was no significant difference in the percentage of PS exposure in SeRBCs between the groups. In contrast, the PS externalization level in YRBCs and the total RBC population was significantly lower vs. Co (*$p < 0.05$; **$p < 0.01$ respectively). The membrane-bound IgG showed no significant differences between AIH patients vs. Co in all populations of RBCs. This study suggests that the decrease in PS exposure observed in YRBCs and total RBCs could be attributed to prednisone and azathioprine which mitigate oxidative and osmotic stress. Both processes are responsible for promoting PS externalization. However, in SeRBCs, the natural aging process may have had a more significant influence than the treatment, allowing them to reach PS exposure levels similar to those of the Co group. Additionally, we observed a decrease in CD47 expression, suggesting increased erythrocyte senescence and shedding light on underlying mechanisms that might contribute to elevated hepatic iron levels in liver pathologies.

57. 723. **ACTIVATION AND REGULATION OF THE COMPLEMENT SYSTEM IN MYASTHENIA GRAVIS**

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Myasthenia gravis (MG) is an autoimmune disease primarily mediated by autoantibodies (autoAbs) targeting the acetylcholine receptor at the neuromuscular junction, leading to muscle fiber destruction via complement activation. While the complement system's role in MG is well-documented in animal models, its contribution in human patients remains unclear. This study provides a comprehensive analysis of complement proteins, regulators, and activity in an Argentinian MG cohort. We analyzed the expression of CD55, CD59, and CD46, three membrane-bound complement regulatory proteins, on leukocytes from 22 MG patients and 10 healthy controls (HC) using flow cytometry. Serum concentrations of C3, C4 (radial immunodiffusion), C5a (ELISA), complement system functionality (CH50 assay), and autoAbs-mediated complement activation were also assessed. Disease severity was measured using ADL and MGC clinical scores, and PISG classification. Our results show lower CD46 expression in MG patients compared to HC (MG MFI: 6608 vs. HC: 8586, $p=0.030$), especially in those with exacerbation (exc MFI: 3705 vs. HC MFI: 8586, $p=0.028$). No significant correlations with severity scales were found. However, CD55 expression was positively correlated with ADL ($p=0.035$, $r=0.4513$) and MGC scores ($p=0.0064$, $r=0.5631$). CD46 expression correlated with disease duration ($p=0.015$, $r=0.5136$). CH50 was reduced in MG patients, but all values remained within normal ranges. No associations were found with other parameters. In conclusion, reduced CD46 expression in severe MG may indicate vulnerability to complement-mediated damage. The observed correlations suggest a compensatory increase in regulatory protein expression to counteract complement activation. Further studies with larger cohorts are needed to validate these findings

58. 725. **THE SEARCH FOR BIOMARKERS IN GLOMERULONEPHRITIS: THE CASE OF CYSTATIN C AND ANTI-C1Q**

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Background: Lupus nephritis affects about 40% of patients with Systemic Lupus Erythematosus (SLE). Despite treatment, only 30% achieve complete remission, and up to 20% progress to end-stage renal disease within 5 years of diagnosis. Currently, renal biopsy remains the gold standard for diagnosis and monitoring, despite the risks and complications involved. Proteinuria has been proposed as a useful biomarker for monitoring LN, but its utility is questioned since up to one-third of patients will persist with "silent" histological activity despite clinical remission. This has motivated the search for new serum or urinary biomarkers for diagnosing and monitoring LN.
Anti-C1q has been proposed as a useful biomarker in the diagnosis, monitoring, and prognosis of LN. Similarly, Cystatin C has emerged as a more stable marker for calculating renal function. **Ob-**

jective: To measure the correlation between the values of Cystatin C, anti-C1q, and proteinuria in lupus nephritis. **Methodology:** Twenty-eight patients with SLE and LN were included for analysis. Categorical variables are described with frequencies and percentages, and numerical variables by means and standard deviation (SD). Correlations were calculated using Pearson's correlation coefficient. **Results:** Of the 28 patients, 86% were women. The average age was 26 (SD 9.4). Of the histological classes, 78% corresponded to classes III and IV. Anti-C1q was positive in 72% of class III, 82% in class IV, and 100% in class IV/V. Cystatin C values were positive in 64% of patients with class III and IV LN, respectively. The correlation index for anti-C1q/proteinuria was -0.28 and for Cystatin C/proteinuria was 0.15. **Conclusion:** The study highlights the potential of anti-C1q and Cystatin C as biomarkers in the diagnosis and monitoring of lupus nephritis. The correlation was weak between these serum biomarkers and proteinuria.

DIAGNOSTIC TOOLS IN IMMUNOLOGY

59. 112. **CLINICAL VALUE OF GENE EXPRESSION PROFILING BY DIGITAL PCR FOR THE DETECTION OF LEUKEMIC CELLS IN PEDIATRIC POPULATION**

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Background: Acute lymphoblastic leukemia (ALL) is the most frequent pediatric malignancy with the gold standard for its diagnosis being immunophenotyping by flow cytometry. Our team recently identified a group of amplified genes in bone marrow samples of pediatric ALL patients through CGH arrays. **Objective:** In this work, we aimed to validate the elevated expression of some of those genes employing digital PCR (dPCR), an emerging technique that has gained relevance in the field for the enhanced sensibility and reproducibility it provides. **Methods:** In short, we analyzed 35 bone marrow samples, immunophenotype (EuroFlow) was determined and RNA was extracted to obtain cDNA. We conducted an initial assessment for the expression of 18 relevant genes using real-time PCR (qPCR), those genes with most notable overexpression in the leukemia group (n=29) compared to individuals without leukemia (n=3) and minimal residual disease (MRD) negative patients (n=3) were evaluated using dPCR. Thus, we measured mRNA expression of *JUP*, *CNP*, *NT5C3B*, *C-MYC* and *BIRC5* in the QIAcuity One instrument obtaining absolute quantification values (number of copies per reaction). Data was analyzed using RStudio and statistically significant differences were established using a Kruskal–Wallis test. **Results:** Our results show that *JUP*, *NT5C3B*, *C-MYC* and *BIRC5* are genes significantly overexpressed at mRNA level in bone marrow samples of leukemia patients in contrast with samples derived from individuals without leukemia and minimal residual disease negative patients, behaving as high sensitivity and specificity markers. **Conclusion:** These genes hold significant potential for detecting leukemia cells in pediatric populations when evaluated as a gene panel using dPCR. This method offers high sensitivity, eliminates the need for calibrator samples, and provides absolute quantification of gene expression at the mRNA level

60. 183. **THE RV2626C ANTIGEN COMPLEMENTS ESAT-6 AND CFP-10 ON DETECTION OF SPECIFIC PLASMA IGG FOR DIAGNOSIS OF TUBERCULOSIS INFECTION**

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Background: Tuberculosis (TB) is still one of the world deadliest diseases, killing around 1.3 million people each year. Its etiologic agent, *Mycobacterium tuberculosis* (*Mtb*), has evolved to withstand the human immune response, displaying distinct antigenic profiles during infection. ESAT-6 and CFP-10 are antigens associated with Mtb proliferation whereas Rv2626c is known to be induced under hypoxic conditions. We have previously found that detection of specific IgG levels against a fusion protein (Fp) of ESAT-6 and CFP-10 showed modest diagnostic potential for TB with AUC: 0,7451; 59,15% sensitivity (95% CI: 50.93% to 66.90%) and 85.07% specificity (95% CI: 74.66% to 91.69%). Objectives: To evaluate a possible synergistic effect of Rv2626c with ESAT-6 and CFP-10 for detection of Mtb infection. Methods: Levels of IgG against Fp and Fp with Rv2626c were measured by ELISA in plasma samples from tuberculosis patients (TB), close contacts (CC) and non-exposed individuals (NE). Results: Addition of Rv2626c to Fp allowed the detection of 40.0% of Fp false negatives, significantly improving the diagnosis with AUC: 0.8555; Sensitivity: 75.29% (95% CI: 65.17% to 83,24%) and Specificity 84.38% (with 95% CI: 68.25% to 93.14%). We have also observed an increase in the percentage of CC with detectable antigen-specific IgG from 44.4% to 72.9%. Surprisingly, we also found that IgG levels against Fp and Rv2626c were elevated in both IGRA-positive and IGRA-negative CC samples. Conclusions: Our findings indicate that Rv2626c effectively complements ESAT-6 and CFP-10 in the serodiagnosis of TB, increasing the percentage of individuals with detectable antigen-specific IgG levels.

61. 185. CIRCULATING SFAS, HE4, CA125 AND APOPTOSIS INDUCTION AS BIOMARKERS FOR THE DIFFERENTIAL DIAGNOSIS OF BENIGN AND MALIGNANT ADNEXAL MASSES

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Background: Ovarian cancer is a leading cause of gynecologic cancer deaths, with 313,000 new cases and 207,000 deaths annually. Currently, few markers are available to distinguish between benign and malignant adnexal masses, leading to many patients undergoing exploratory laparotomy. However, only one-third of these patients are ultimately diagnosed with a malignant tumor. Therefore, it is important to continue researching tests or markers that can differentiate both diagnoses.
Objective: To evaluate the usefulness of serum markers (sFAS, CA125, HE4 and the apoptosis induction in Jurkat cells), to differentiate between benign and malignant adnexal masses **Methods** Sera from patients with benign tumors (n=42), malignant tumors (n=36), and healthy donors (n=9) were analyzed for soluble molecules (HE4/CA125 by ELISA; sFas by flow cytometry-Legendplex). Jurkat cells were cultured with 200 ul patient serum, and apoptosis was measured after 24 hours using flow cytometry (PI/Annexin V). Statistical analysis was conducted using ANOVA followed by Tukey's post hoc test, with significance levels set at $p < 0.05$. **Results** Sera from malignant tumor patients tended to induce less apoptosis in Jurkat cells compared to benign adnexal masses and healthy controls, which had the highest apoptosis percentages, whereas no differences were observed in the levels of sFas. HE4 and CA125 serum levels was significantly higher in ovarian cancer patients compared to those with benign adnexal masses and healthy controls. **Conclusion** HE4 levels were most effective in distinguishing benign from malignant adnexal masses, with elevated levels observed in ovarian cancer. Sera from malignant cases induced less apoptosis in Jurkat cells, suggesting their potential in assessing tumor behavior. Together, these biomarkers could enhance ovarian

cancer diagnosis and improve early detection.

62. 273. **IFN-GAMMA RESPONSE TO MYCOBACTERIUM TUBERCULOSIS SPECIFIC ANTIGENS DIFFERENTIATES LATENT TUBERCULOSIS INFECTION IN CHILDREN**

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Background. *Mycobacterium tuberculosis* (*Mtb*) infects millions of children every year, and most cases are never diagnosed or treated. Pediatric tuberculosis (TB), similar to adult TB, presents as active TB and latent TB infection (LTBI). LTBI children display no clinical or radiologic signs of disease, but they are at high risk of progressing to active TB, especially children under five years old. IFN-γ release assays (IGRAs) are better indicators of *Mtb* infection as compared to tuberculin skin test in Bacillus Calmette–Guérin (BCG)-vaccinated populations. However, IGRAs do not discriminate active and LTBI. Furthermore, no gold standard for LTBI diagnosis is currently available. **Objective.** To assess the efficacy of *Mtb* antigens, secreted during the active and latent stages, to diagnose *Mtb*-infected children. **Methods.** BCG-vaccinated healthy controls (HC), contacts of active TB (CC) and TB patients between 0-14 years old were recruited. Peripheral blood mononuclear cells were isolated and stimulated at 2.5 µg/ml with each *Mtb* antigen (CFP-10-ESAT-6 fusion protein (PF), HspX, Rv2626c, Rv2628 or Rv3716c) during 5 days. IFN-γ production was tested in culture supernatants by ELISA. **Results.** IFN-γ levels produced against PF allowed to differentiate TB patients from HC ($p<0.0001$, Mann-Whitney test). Accordingly, ROC analysis indicated 92.9% sensitivity, 100% specificity and a cut-off =20pg/ml. Moreover, considering the cut-off value, we identified two groups of individuals among CC subjects: $CCPF^+$ (>20pg/ml) and $CCPF^-$ (<20pg/ml). Interestingly, $CCPF^+$ individuals produced significantly higher IFN-γ levels against Rv2626c, Rv2628 and HspX than HD, $CCPF^-$ and TB ($p<0.05$, Mann-Whitney test). Finally, the IFN-γ secreted against Rv3716c by $CCPF^+$ and TB resulted strikingly higher than the levels produced by HC ($p<0.05$, Mann-Whitney test). **Conclusions.** Cell stimulation with the specific *Mtb* antigens studied allowed to detect *Mtb*-infection in the pediatric population. Further studies using proper antigen combinations might improve LTBI diagnosis, even in a BCG-vaccinated population.

63. 309 **IMPROVING THE SEROLOGICAL DIAGNOSIS OF ECHINOCOCCUS GRANULOSUS**

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Background: The serological diagnosis of *Echinococcus granulosus* relies on detecting patient antibodies that react with parasitic antigens. The immune response can vary, and antibodies may persist post-treatment. Although histone H4 from *Echinococcus granulosus* is recognized by patient sera, its first 103 amino acids are shared with other organisms like *Fasciola hepatica*. Thus, specific detection of circulating antigens using carefully designed antibodies may more accurately indicate the presence of the parasite. **Objectives :** Analyze the antigenic properties of histone H4 from *Fasciola hepatica*. **Methods:** Ab initio modeling was performed in Robetta platform. The quality of the model was analyzed with Ramachandran plot and ERRAT. The molecular dynamics simulation (MD) validated the model using the NAMD2 (CHARMM36 force field, standard number of particles, 1 atm, explicit solvent, NaCl 0.15 M, periodic boundary conditions). The simulation protocols involved: 2000 steps of minimization; 0.24 ns of heating 60K -300K; 1 ns equilibration at 300K; and unrestrained production of 50 ns (300K). The total potential energy and the root mean-square deviation (RMSD)

were calculated in the unrestrained production. The structure was analyzed and visualized with VMD 1.9.3, MOE and mdtraj. The B-cell conformational epitopes for the 50 ns structure were predicted with DiscoTope 2.0 (threshold= -3.7). **Results:** A high-quality three-dimensional model was achieved. The model reached thermodynamic and structural equilibrium, reaching average values of potential energy -89289.97 ± 139.55 Kcal/mol and average RMSD: 4.429 +/- 0.017 (A) in the unrestrained production. Prediction of conformational epitopes was positive for amino acids 1-22, 25, 28-29, 31-34, 36-37, 40-41, 75-81, and 102, with several of these epitopes being shared with *Echinococcus granulosus*. **Conclusion:** We obtained a validated molecular model of histone H4 from *Fasciola hepatica*, which enables the design of antibodies that can differentially recognize histone from *Echinococcus granulosus* and *Fasciola hepatica*.

64. 374. **NANOBODY-BASED DIAGNOSTIC TOOLS FOR IGE AND OMALIZUMAB DETECTION IN SEVERE ASTHMA TREATMENT**

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Biological therapy with Omalizumab is indicated for patients with severe asthma that does not respond to conventional corticosteroid treatments. Omalizumab is a monoclonal antibody that mitigates allergic reactions by binding to human IgE and blocking its interaction with the high-affinity FcεRI receptor on basophils and mast cells. In Uruguay, treatment response is primarily assessed through conventional clinical questionnaires without additional blood marker measurements. However, treatment regulation could be optimized by measuring serum levels of free IgE (unbound to the drug) and total IgE (both free and drug-bound), as well as drug levels during treatment. This work proposes the development of diagnostic tools based on single-chain antibodies (nanobodies). To detect IgE, we have generated chimeric IgE proteins composed of the CH3, CH3-CH4, and CH2-CH3-CH4 Fc domains, which are used as immunogens for nanobody discovery and as standards in immunoassays. Additionally, we have obtained anti-IgE nanobodies that enable IgE detection and have been categorized into three distinct families based on their IgE recognition epitopes. For Omalizumab detection, we developed two groups of anti-idiotype nanobodies with non-overlapping recognition epitopes. By pairing these nanobodies, we can perform highly specific sandwich immunoassays to measure the drug. In summary, we have designed a panel of anti-IgE and anti-Omalizumab nanobodies that facilitate the detection of total IgE and the drug in blood, respectively. Our preliminary findings guide us toward developing novel strategies for identifying nanobodies capable of recognizing free IgE, with a specific focus on epitope overlap with Omalizumab. Additionally, the availability of anti-idiotype nanobody specific to Omalizumab will enable the implementation of 'mix and read' immunoassays, which detect the analyte in solution when sufficient proximity between reagents enables signal generation. Ultimately, these tools will facilitate the integration of IgE and Omalizumab detection into a single multiplex assay.

65. 418. **COOPERATIVE ADSORPTION OF ANTIBODIES ON GOLD NANOPARTICLES: A NOVEL APPROACH FOR NANOTECHNOLOGY-ENABLED BIOSENSORS**

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Background: Antibodies such as Rituximab and Infliximab are pivotal in immunotherapy, targeting a range of diseases from cancer to autoimmune disorders. The development of new antibodies, such as anti-sST2 (ulcerative colitis) and anti-MICA (gastric cancer), offers new opportunities to enhance treatment and diagnostics. The growing interest in nanomedicine, particularly in using gold nanoparticles, is due to their unique properties, including ease of functionalization, biocompatibility, and stability. This study examines the efficacy of a cooperative adsorption technique to conjugate different antibodies to gold nanospheres without altering the nanoparticle or antibody structure, facilitating the development of novel biosensors. **Objectives:** To demonstrate the versatility and effectiveness of the cooperative adsorption protocol in conjugating various therapeutic antibodies (anti-sST2, anti-MICA, Rituximab, Infliximab) to gold nanospheres, aiming to establish a robust method for creating biosensors and therapeutic delivery systems. **Methods:** Gold nanospheres were functionalized using a cooperative adsorption technique with four different antibodies and subsequently blocked with BSA. The nanosystems were characterized by

measuring hydrodynamic diameter, Z-potential, electron microscopy, and UV- Vis spectrophotometry. Antigen recognition capabilities were assessed using SDS-PAGE or Western blot assays. **Results:** Consistent results were obtained across all antibodies, with gold nanospheres exhibiting a hydrodynamic diameter of 59.8 ± 26.9 nm, a Z-potential of -32.6 ± 13.5 mV, and a STEM diameter of 41.1 ± 4.1 nm. Successful antibody conjugation and BSA blocking were confirmed by an increased hydrodynamic diameter (100 ± 29.8 nm) and a UV-Vis plasmon red shift (3-6 nm) for Infliximab, Rituximab, anti-sST2, and anti-MICA. Antigen recognition was detected for Infliximab, anti-sST2, and anti-MICA.
Conclusion: The cooperative adsorption technique effectively conjugates various therapeutic antibodies to gold nanospheres, preserving their functionality. This method holds significant potential for developing versatile biosensors and therapeutic delivery platforms, applicable to multiple pathologies.

66. 462. **IDENTIFICATION OF A SPECIFIC NS1-DENV EPITOPE TO SERVE AS A TOOL FOR THE SEROLOGICAL DETECTION OF THE FOUR DENGUE SEROTYPES**

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Dengue is a viral disease whose infection can lead to the development of a serious illness and even death. In regions where multiple arboviruses co-circulate, the differential diagnosis of dengue is challenging due to the potential for cross-reactivity of serum antibodies with other flaviviruses. This issue is exacerbated by the absence of safe and widely available vaccines. Therefore, it is essential to develop new immunobiological tools that can specifically target the dengue virus and its variants. This work aimed to identify an optimized epitope of the non-structural protein 1 (NS1) of dengue virus (DENV) that is conserved across all four dengue serotypes and does not exhibit homology with other flaviviruses, making it a suitable target for antibody selection in diagnostic assays. A multiple sequence alignment of the NS1-DENV peptide regions described as specific and sensitive was performed. On the I-TASSER bioinformatics platform, the ideal three-dimensional structures of these regions were constructed and analyzed for their biochemical properties. The optimized gene was synthesized, cloned into the pET28a vector, and expressed in *Escherichia coli* BL21 (DE3) cells. After expression, the protein was purified by immobilized metal affinity chromatography (IMAC) and analyzed by SDS-PAGE. Recognition of the polypeptide was confirmed by ELISA using anti-His tag and anti-NS1 monoclonal antibodies. The study identified two potential epitopes as promising targets for antibody-based dengue detection, with reduced chances of cross-reactivity with other flaviviruses. Once these tools are obtained, new possibilities for the development of more accurate detection tests and efficient therapeutic strategies can be explored and contribute to the surveillance, adequate treatment and prevention of dengue.

67. 571. **DEVELOPMENT OF AN ELISA KIT FOR THE DETECTION OF IGA ANTI-TISSUE TRANSGLUTAMINASE ANTIBODIES USED IN THE DIAGNOSIS AND MONITORING OF CELIAC DISEASE**

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Celiac disease is currently considered to be the most common chronic bowel disease. An approximate global prevalence of 1-2% has been estimated. The IgA isotype of anti-tissue transglutaminase (a-tTgA) is the most sensitive serological marker and is routinely used in the diagnostic algorithm proposed by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). These antibodies are determined by ELISA kits that are imported, making the

product expensive and sometimes in short supply. The aim of this work was to develop and validate a national ELISA kit for the detection of a-tTgA antibodies for the diagnosis and monitoring of celiac disease. Recombinant tTg was produced and sensitised on 96-well plates. Serum samples from volunteers with different levels of a-tTgA, determined by commercial kits in external laboratories, were incubated. Peroxidase- conjugated anti-human IgA antibodies were added, then TMB was used as a reaction substrate and read at 450 nm. The calibration curve was constructed from dilutions of positive sera. The concentrations of tTg, serum and conjugated antibody, number of washes and incubation times for each step were optimised. Once these parameters had been fine- tuned, validation was performed with negative, low, medium and high positive sera. Intra- and inter-assay precision, specificity and sensitivity were calculated. In addition, the performance of the developed kit was tested against the standards of a commercial kit. The intra-assay coefficient of variation ranged from 0.3 to 8% and the inter-assay coefficient of variation ranged from 4 to 18%. The specificity of the developed ELISA was 94.5% and the sensitivity 96.8%. The goodness of fit with the commercial kit standards showed a regression R of 0.998, indicating that the antibodies used in the kit recognise the recombinant tTg used in our kit. In conclusion, an ELISA kit for the detection of a-tTgA has been successfully developed and validated.

68. 614. **PODOCYTE-DERIVED EXTRACELLULAR VESICLES ASSOCIATED WITH IMMUNE MEDIATORS AS POSSIBLE BIOMARKERS OF KIDNEY DAMAGE IN THE CONTEXT OF LUPUS NEPHRITIS**

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Background: Lupus nephritis (LN) affects 40-75% of systemic lupus erythematosus (SLE) patients. LN is characterized by podocyte injury, and the detection of urinary extracellular vesicles (uEVs) may suggest the onset of podocyte damage and renal inflammation. Objectives: To evaluate uEVs as potential biomarkers of kidney injury in SLE patients. Methods: A cross-sectional study was carried out with SLE patients and healthy donors (IRB#60747722.2.0000.5243). SLE and kidney activity were measured using the SLEDAI-2K (cut-off point ≥5) and the R-SLEDAI index (cut-off point ≥4), respectively. The first-morning midstream urine was collected and immediately processed (2,000xg 5 min). After thawing, uEVs were isolated by differential centrifugation and quantified by nanoscale flow cytometry (nFC) and nanoparticle tracking analysis (NTA). In nFC, we considered a size of ~100-900 nm, positivity for Annexin V and Anti-human podoplanin. Urinary immune mediators were quantified using a multiplex assay. The Mann-Whitney or Kruskal Wallis tests were used to analyze the study groups. Correlation analysis using the Spearman coefficient. Results: We studied 82 SLE patients (42.6±11.3 years, 91.4% female), with a disease diagnosis time of 11.2±7.0 years and 56.1% with LN, 18 volunteers without autoimmune disease (37.5 ±8.2 years, 83.3% female). In NTA, no differences in the number of particles/mL and medium diameter (nm) were found between the groups. However, we observed higher levels of total and podocyte-derived uEVs (P=0.02; P=0.01) in patients with SLE by nFC. Interestingly, patients with active SLE and R-SLEDAI ≥4 presented higher counts of podocyte uEVs (P=0.008; P=0.06, respectively). Also, patients with active LN were associated with higher urinary levels of IL-1β, IL-6, and CCL-2 (P=0.0001; P=0.01; P=0.003, respectively) were also found in this group. Lastly, we identified that urinary IL-5, -8, -9, -10, -12, and GM-CSF were significantly correlated with podocyte-derived uEVs in active LN (p<0.05). Conclusions: These findings suggest podocyte-derived uEVs are associated with SLE activity and active LN, probably reflecting renal inflammation.

IMMUNODEFICIENCIES / AUTOINFLAMMATION

69. 160. **WILD SYNDROME (WARTS, IMMUNE DEFICIENCY, LYMPHATIC-DYSPLASIA) ASSOCIATION WITH A VARIANT OF PLCG2.**

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BACKGROUND: WILD syndrome (2008, Kreuter et al), diagnosis requires the presence of primary lymphedema before 2 years of age, and two of: cutaneous lymph vascular malformation; genital edema; recurrent warts; CD4 lymphopenia and systemic involvement (pleural or pericardial effusion); in the absence of: low monocytes, family history of similar malformations and segmental overgrowth with discrepancy in limb length. . It is related to variants in GATA2 gene. CASE REPORT: We present a 22 years old female with multisegmentary primary lymphedema (lower limbs and genitals) and swelled genitals at birth; uterus didelphys. Otomastoiditis at 4 years old, with hospitalization. Recurrent respiratory infections requiring antibiotics. Thrombocytopenia and hypogammaglobinemia since childhood. Bone marrow aspiration: macroplatelets, anisoplateletosis (ages 10 and 14). Recurrent episodes of erysipelas on the inner thigh. Isolated outbreaks of vesicular-papular lesions in legs, histologically kerato-acanthomas. Cold induced urticaria without fever. Laboratory: lymphopenia, low CD4+ and CD8+; low B-lymphocytes (transitional and total memory), low gammaglobulin, low IgA, G and subclasses. Thrombocytopenia; absence of autoantibodies. Exome sequencing (CentoXome® Solo) retrieved a variant of uncertain significance (VUS) in phospholipase C gamma-2 (PLCG2 c.-2_1del) gene. PLCG2 is a critical signaling molecule, which dysfunction is associated with cancer, neurodegeneration, and immunological disorders (antibody deficiency, immune deregulation, and autoinflammation). In embryonic life, PLCG has a role in separation of lymphatic and blood vessels after its sprouting, which dysfunction causes primary lymphedema. Also associated with: autoinflammation (APLAID), antibody deficiency (CVID) and lymphedema with antibody deficiencies (WILD syndrome), the latter, like the patient's phenotype. Subcutaneous G immunoglobulin therapy achieved hematologic, infectious, lymphatic and dermal improvement.

70. 176. **HOMOZYGOUS RFXANK MUTATION IN ARGENTINEAN PATIENT WITH MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II DEFICIENCY: A CASE REPORT**

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Background: Combined immunodeficiencies are a diverse set of primary immune disorders characterized by T-cell and B-cell function deficiencies. Major histocompatibility complex class II (MHC-II) deficiency is a rare, early-onset, autosomal recessive, and life-threatening inborn immunity error. MHC-II deficiency results from gene mutations encoding transcription factors essential for MHC-II expression, including RFXANK. **Objective:** to characterize the genetic profile of an Argentinean patient with suspected MHC-II deficiency. **Case report:** A 10-year-old male, born in Argentina to consanguineous parents with recurrent infections, underwent clinical, immunologic, and genetic evaluation, including whole exome sequencing. The patient presented oral candidiasis, chronic diarrhea, and failure to thrive. Immunological assessment revealed IgA deficiency, decreased memory and post-switch B-cells, CD4 lymphopenia, and low expression of HLA-DR in B-cells and monocytes. Whole exome sequencing analysis uncovered biallelic mutations in RFXANK (c.338-25_338delGGTATTGCCCGCCTCCTCCTGCCAGG, p.Gly113fs), a key regulator of MHC-II transcription. This frameshift mutation was classified as pathogenic according to ACMG criteria. Population frequency analysis revealed its rarity in GnomAD 4.0, with minimal occurrences in the global population, notably with 11 out of 24 alleles observed in the admixed American population. **Conclusions:** Our findings expand the understanding of MHC-II deficiency, emphasizing the significance of RFXANK mutations in this disorder. Genetic counseling for consanguineous families in Argentina can benefit from our immunological and genetic insights. Further investigations are warranted to determine the prevalence of RFXANK mutations in the Argentinean population and improve for MHC-II deficiency.

71. 383. **SUBCLINICAL INTESTINAL INVOLVEMENT IS RELATED TO A REDUCTION IN SHORT-CHAIN FATTY ACIDS AND METABOLIC PATHWAYS IN PATIENTS WITH SPONDYLOARTHRITIS**

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Background and Aims. Dysbiosis is an environmental factor that affects the induction of spondylarthritis (SpA) and contributing to the alteration of short-chain fatty acid (SCFA) synthesis.The aim was to compare SCFA levels and fecal microbiome composition in SpA patients versus controls. **Methods:** 24 adults were included,12 with SpA without Inflammatory Bowel Disease-IBD, 6 with IBD (dysbiosis) and 6 healthy (eubiosis) control (HC). Patients were evaluated for the presence of gastrointestinal symptoms (GS). Quantification of SCFAs in feces was performed by ultra-high pressure liquid chromatography coupled to mass spectrometry. The 16S rRNA gene were prepared and amplicon sequencing (MiSeq) was carried out for microbiome analysis and in silico inference of metabolic pathways. **Results:** Significant differences in fecal butyric acid levels between the study groups were found. Decreased levels of butyric acid and Total-SCFA were observed in SpA with GS and IBD compared to HC. Butyric acid in the HC and the SpA without GS showed significant differences compared to the IBD. Metabolic predictions showed significant differences in vitamin biosynthesis pathways between all groups analyzed. A significant reduction of pathways related to fatty acid biosynthesis was observed in IBD, compared to SpA with GS. Alpha and beta diversity did not show differences between groups. Taxonomic analysis showed an increase in the phylum Tenericutes and the species *Coprococcus eutactus* in SpA patients with GS. Protective species such as *Alistipes finegoldii* and *Lactobacillus ruminis* showed a significant reduction in IBD. **Conclusions:** Subclinical intestinal involvement in patients with SpA was related to a reduction in butyric acid. A differential profile observed in SCFA and some metabolic pathways discriminates SpA patients with or without GS but the diversity and richness of the fecal microbiome not changed. The role of vitamins and SCFA in regulating the intestinal health immune response is well known as such disease activity.

72. 397. HEREDITARY ANGIOEDEMA WITH C1 INHIBITOR DEFICIENCY: EXPERIENCE IN PEDIATRIC PATIENTS

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Hereditary angioedema with C1 inhibitor deficiency (C1-INH-HAE) is a rare autosomal dominant disorder due to either deficiency (type I) or dysfunction (type II) of the serine protease C1 inhibitor. Clinically it is characterized by recurrent episodes of skin-mucosal, non-pruritic, burdensome and potentially life-threatening edema. Often it presents in childhood. The function y/o concentration of C1–INH are low, C4 levels are usually low and C3 are normal. OBJECTIVES: To describe clinical and laboratory findings in 10 C1-INH-HAE pediatric patients (p). METHODS: retrospective review of medical records RESULTS: 6 males. 9p C1-INH type I, 1 type II. Mean follow up: 7, 5 years (range: 3-13). 6p index case, 4p detected by family screening. 7p with other affected relatives. 3p asymptomatic. Mean age of onset: 3,5 y (r: 1-6). Mean age to diagnosis: 7 y (r: 5-10). First clinical manifestation: 3p abdominal pain (2/3 plus recurrent extremities edema and erythema marginatum-1/3p plus vomiting), 2p face angioedema, 1p recurrent extremities edema and erythema marginatum. Misdiagnosis: allergic edema/angioedema, 2p arthritis, 1p

acute appendicitis, 1p figurative urticarial. Patients were referred from pediatrics, rheumatology, dermatology and allergy. 3p had laryngeal episodes, 2/3 more than one, none had long-term prophylaxis. 3/10 had had surgeons without treatment. 6p received specific treatment for the crisis and 2/7 long-term specific prophylaxis with good results. Mean C4: 6 mg/dl (VN 15-35), mean C1-INH (9/10p): 5 mg/dl (VN 19-39). CONCLUSIONS: -The clinical onset was at early age, arriving later at diagnosis. -Despite of abdominal symptoms, none of them were referred from gastroenterology. -Is to be noted the high number of laryngeal episodes in our cohort. -Early diagnosis and treatment besides family screening could prevent severe complications. - The C1-INH and C4 levels plus clinical data are highly suggestive of C1-INH-HAE.

73. 422. **CASE REPORT: SPONTANEOUS DOCK8 T CELL REVERSION IN A PATIENT WITH HIPERIGE SYNDROME**

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Background: Biallelic loss-of-function mutations in the Dedicator of Cytokinesis 8 (*DOCK8*) gene leads to HiperIgE syndrome, a combined primary immunodeficiency disease characterized by susceptibility to viral infections, recurrent infections, atopy, malignancies and autoimmunity. Somatic reversion of the pathogenic variant has been reported in a few patients. **Objective:** present the clinical, immunologic and molecular features of a patient with DOCK8 deficiency. **Results:** 13-year-old boy(yo), born from non-consanguineous parents. Full-term baby with no relevant prenatal history. History of multiple hospital admissions due to broncho-obstructive syndrome and pneumonia since 3 months old. Also, recurrent bilateral suppurative acute otitis media and atopic dermatitis with a regular response to treatment that required several cycles of corticosteroids and antibiotics. Prolonged febrile syndrome with polyserositis with antibiotic and anti-inflammatory drugs requirement. He also developed several ischemic stroke events confirmed by RMI. Laboratory: hypereosinophilia, normal IgG, low IgM and high IgA and IgE levels. Impaired specific polysaccharide response. T cell lymphopenia with increased CD4+ and CD8+ memory cells with activated phenotype. Low class-switched memory Bcells and expansion of transitional Bcells. Additional studies showed impaired NK cells cytotoxicity, normal T cell lymphoproliferative response; high follicular helper T cells (Tfh) with skewed toward Tfh1 and increase IL-4 cytokine production including conventional memory Tcell Th2. Molecular studies revealed compound heterozygous *DOCK8* pathogenic variants: NM_203447.4:c.2109+4A>G/rsa[GRCh36] 9p24.3 (204804_261711)x1. Absence of DOCK8 expression in NK and B cells and bimodal expression in CD4+ (17%) and CD8+ (36%). He received a hematopoietic stem cells transplant. At day +180, he has no immune reconstitution yet. Nowadays he continues under gammaglobulin, profilactic antobiotics and antivirals treatment. **Discussion:** We present a patient with DOCK8 deficiency and bimodal protein expression suggesting spontaneous restore in T cells. Although *DOCK8* Tcell reversion is associated with less severe phenotype according to the bibliography, our patient did not evidence milder clinical outcome.

74. 429. **CONGENITAL NEUTROPENIA: CASE REPORT**

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Severe congenital neutropenia is characterised by a neutrophil count below 0.5 x109 l, determined by a primary failure of myelopoiesis. It presents a great genetic heterogeneity, the most frequent mutations are found in the Elastase 2 gene. It is represented by severe bacterial infections from a very early age, with absence of pus as a characteristic feature. The treatment of first choice is the administration of granulocyte colony stimulating factors. Male, 8 months old, born at term, third child, denies consanguinity. History of omphalitis, becegeitis, cellulitis and impetigo in the neonatal period. Evaluated for recurrent suppurative otitis media and urinary tract infection. Negative serology. Laboratory with persistent neutropenia and anaemia. Decreased total B lymphocytes, with increased memory with switched and decreased CD4+ and CD8+ T lymphocytes at the expense of naive. Normal immunoglobulins. Cranial CT scan showing a lytic lesion in the left mastoid, suspected histiocytosis. Bone mapping and scintigraphy were normal. Mastoid biopsy showed bone tissue without malignancy, focal chronic inflammation. Bone marrow puncture showed arrested

maturation of the myeloid series, predominantly promyelocytes. Treatment with granulocyte stimulating factor was started without response. Genetic study found a probably pathogenic mutation in the ELANE gene encoding a neutrophil elastase protein, associated with severe congenital neutropenia. Due to refractoriness to treatment, a bone marrow transplant was requested as an alternative therapy in this entity.

75. 434. **SELECTIVE IGA DEFICIENCY DURING CELIAC DISEASE SCREENING**

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Background: The incidence of celiac disease (CD) in individuals with selective IgA deficiency (SIgAD) is higher than in healthy populations. Due to the common immunopathogenic mechanism, the presence of SIgAD becomes a risk factor for CD, showing high co-occurrence in patients. Objectives: The aim of this research was to study the co-occurrence of SIgAD and positive serology of CD in a Uruguayan population. Furthermore, it sought to determine the threshold value of IgA which needs further testing for anti-transglutaminase IgG antibodies (anti-tTG IgG) in the screening of CD, when IgA levels are between undetectable (< 7 mg/dL) and the minimum normality range. Methods: An observational and retrospective study was made, including samples from patients older than 4 years, referred to the Clinical Pathology Department of the Pereira Rossell Hospital Centre, from January 2020 to July 2024 for diagnosis or follow-up of CD. Anti-tTG IgG testing was performed due to reduced IgA levels. Results: Among the total population studied for CD screening by the laboratory during that period (17,582 individuals), the incidence of SIgAD was 0.8%. In our selected population of 234 patients, 63% (147) had SIgAD. The frequency of individuals with positive CD serology who simultaneously had SIgAD was 0.80 (33 of 41). Male individuals reported a significant difference in that frequency, being 0,89 (16 of 18), while 0.74 (17 of 23) corresponded to female individuals (p=0.004). Conclusion: An intermediate range exists between the diagnostic criterion for SIgAD and the lower limit of normality for IgA, in which the anti-tTG IgG test may be necessary to detect CD. Positive anti-tTG IgG results were found in both children and adults with IgA levels lower than 20 mg/dL. This threshold suggests a possible criterion for implementing IgG antibody tests, which could improve the accuracy of CD screening.

76. 532. **IMPACT OF LONG-TERM PROPHYLAXIS ON SHORT-TERM PROPHYLAXIS REQUIREMENTS IN PATIENTS WITH HEREDITARY ANGIOEDEMA**

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Introduction: Hereditary angioedema (HAE) is a condition characterized by recurrent episodes of edema affecting subcutaneous and submucosal tissues. Significant advancements in treatment have been made, and long-term prophylaxis (LTP) is now available, normalizing patients' lives. However, there is a gap in evidence regarding the need for short-term prophylaxis in patients receiving LTP who undergo surgical procedures. The latest WAO/EAACI consensus guidelines for managing HAE encourage the publication of evidence on this topic.**Objective:** To provide evidence on whether short-term prophylaxis is necessary for patients with HAE who are on long-term prophylaxis. **Materials and Methods:** A retrospective, observational, and descriptive study was conducted on a cohort of 177 patients managed by a multidisciplinary HAE program based in Bogotá, Colombia. Medical records from August 2022 to July 2024 were reviewed to find evidence supporting the need for short-term prophylaxis in patients receiving LTP. **Results:** Out of the 177 patients, 17 reported undergoing invasive procedures. Of these, 30% were on long-term prophylaxis with lanadelumab; none of these patients received short-term prophylaxis, and none experienced postoperative crises. Among the remaining 70% who were not on LTP, 41% received short-term therapy, and only 0.4% experienced crises. **Conclusions:** This report provides evidence suggesting that short-term prophylaxis may not be necessary for patients on LTP. Similar to the WAO/EAACI consensus for managing HAE, we encourage the scientific community to continue research to enhance recommendations in future management guidelines.

77. 558. **FUNCTIONAL CHARACTERIZATION OF NOVEL PTPN2 VARIANTS IN A PEDIATRIC PATIENT WITH POLYAUTOIMMUNITY AND POLYINFLAMMATION**

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Protein Tyrosine Phosphatase Non-Receptor Type 2 (PTPN2) is a crucial negative regulator of the JAK/STAT signaling pathway, particularly in immune responses. Mutations in PTPN2 have been associated with monogenic autoimmune diseases, highlighting its role in maintaining immune homeostasis. In an Argentinean pediatric patient with polyautoimmunity and polyinflammation, exome sequencing revealed two novel heterozygous variants in PTPN2: c.515T>C (p.Ile172Thr) and c.865T>C (p.Trp289Arg). These variants segregate with the disease within the patient's family, with each variant inherited from a healthy heterozygous parent. To explore the impact of these novel PTPN2 variants, the wild-type (WT) PTPN2 cDNA was cloned into a mammalian expression plasmid tagged with HA. Site-directed mutagenesis was employed to introduce the c.515T>C and c.865T>C variants into the PTPN2 sequence. The WT and mutant PTPN2 constructs were transfected into HEK293T cells, followed by gene expression analysis using Western blotting. Additionally, co-transfection of PTPN2-HA WT or mutated, a WT STAT3 expression plasmid, and a STAT3-responsive GFP reporter was performed. The functional impact of the PTPN2 variants on STAT3 activity was assessed following IL-6 stimulation. Western blot analysis revealed that the expression levels of the mutant PTPN2 proteins were comparable to the WT, indicating no significant alteration in protein stability. However, functional assays suggest that the novel PTPN2 variants enhance STAT3 functionality when present in a compound heterozygous state (n=2 with 3 internal replicates per experiment). Although preliminary, these results indicate a potential effect on STAT3 signaling, consistent with the patient's autoimmune and inflammatory phenotype. These findings suggest that the novel PTPN2 variants identified in this patient may contribute to the dysregulation of STAT3-mediated signaling pathways, leading to polyautoimmunity and polyinflammation. Further investigations are required to confirm these initial observations and to understand the broader implications for targeted therapeutic strategies.

78. 568. **WHOLE EXOME SEQUENCING IN ARGENTINE PATIENTS WITH INBORN ERRORS OF IMMUNITY: A COMPREHENSIVE STUDY**

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Inborn Errors of Immunity (IEI) encompass a wide array of immune deficiency disorders caused by genetic mutations that impact the development and function of the immune system. The identification of causative genes for IEI remains a significant challenge due to genetic complexity and phenotypic variability, even with advancements in molecular genetics and next-generation sequencing technologies. This study aims to enhance genetic diagnosis in Argentine patients with IEI through comprehensive exome data analysis. Over the past year, in collaboration with the professionals of Association of Clinical Immunologists of Argentina (AINCA), we performed whole exome sequencing (WES) on 200 Argentine patients diagnosed with IEI from various hospitals across the country. The bioinformatic pipeline included processing fastQ files, variant prioriti-

zation, and clinical phenotype correlation. The analysis focused on coding exons, exon-intron junctions, and copy number variations (CNVs) in genes associated with immune function. Our findings revealed pathogenic or likely pathogenic variants in IEI-related genes in 21% of the patients. Additionally, 33% of the patients harbored sequence variants in IEI-related genes with unclear significance. Furthermore, 21% of patients carried variants in novel candidate genes for IEI. Notably, 83% of these variants were located in coding regions, with 19% in regulatory regions, CNVs, or genes associated with neurological defects or epigenetic regulation. Currently, we are confirming these variants and conducting family segregation analyzes to further validate our findings and perform family genetic counseling. This study underscores the critical importance of detailed variant analysis in understanding the heterogeneity of monogenic immune defects. The findings highlight the unique roles of specific genes and proteins in the development and function of the host immune system, contributing to more accurate diagnoses and potentially guiding targeted therapeutic interventions.

79. 583. **EXTENDED LYMPHOID PROFILE DESCRIPTION BY FLOW CYTOMETRY IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY**

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Background: Common Variable Immunodeficiency (CVID) is the most common symptomatic Inborn Immune Error (IEI), with heterogeneous clinical manifestations (recurrent infections, autoimmunity, granulomas, lymphoproliferation). A causative genetic defect is identified in only 10-20% of cases, so the diagnosis is based on the European Society for Immunodeficiencies (ESID) criteria, which include a reduction in switched memory B cells. In addition, the T-cell maturation profile is important for follow-up and prognosis. **Aim:** To describe the lymphocyte subpopulations of B cells, CD4+ T cells, and CD8+ T cells in patients with CVID. **Methods:** Observational, cross-sectional study. Clinical data and peripheral blood samples were collected for analysis by flow cytometry to identify lymphocyte subpopulations in 15 patients diagnosed with CVID according to ESID criteria. **Results:** The clinical presentation of these patients is varied: 100% presented with recurrent infections, 40% with autoimmunity, and 7% with autoinflammation. The most frequently altered lymphocyte subpopulations were switched memory B cells (87%), naïve CD4+ T cells (80%), and non-switched memory B cells (73%), as described in the literature. In addition, increased effector memory CD8+ T cells and terminally differentiated memory CD8+ T cells were observed in 40% of patients, reflecting a chronic activation profile that may be related to recurrent respiratory infections. **Conclusion:** CVID is a heterogeneous disease, and its diagnosis is a challenge in which flow cytometry plays an important role. The study of the extended profile emphasizes the importance of assessing each patient individually, contributing to a better understanding of the associated immunological features, and thus improving the understanding of future complications, long-term prognosis, and clinical management of these patients.

80. 598. **EFFECT OF KAÑIWA, QUINOA AND AMARANTH ON THE INMUNE SYSTEM IN MALNOURESHED MICE**

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This work arises from the need to seek new food sources in order to try to control the malnourished problem that affected some vulnerable groups such as children under five years old. It is already known that one consequence of the mention problem is the depletion of inmune system. Several andean grains are being reevaluated for their nutritional properties as kañiwa, quinoa y amaranth. We evaluated the nutritional reflected the richness in Minerals (Iron, Calcium, Phosporus and Zinc), Vitamins (Thiamine, Niacin, Riboflavin, Ascorbic Acid), essential proteins and fatty acids. These nutrients have been shown to improve the immune system. The effectiveness of immune system recovery was evaluated in malnourished mice using kañiwa flour, quinoa and amaranth. For nutritional assessment, the experimental animals were fed a nutrient-poor diet based on corn starch (75%) and cream of milk (25%), then recovered by ingestion of a diet based on kañiwa flour (75%) and cream of milk (25%). Likewise for quinoa y amaranth. The following variables were recorded: body weight (BW), spleen weight (SW), hematocrit (Hto) and hemoglobin (Hb), white blood cell count (WBC). Colorations of the bone marrow (BM) and spleen (S) were observed. Av-

erages, standard deviation, and the Student test were determined.
The recovery of WB, WS, WBC, Hto and Hb was 100% ($p<0.01$). Recovery of hematopoietic cell lines decreased by nutrient deficiency was observed in both spleen and bone marrow. Results apply to kañiwa and quinoa. Not to amaranth. Therefore, kañiwa and quinoa would represent good alternative food, recommended to improve the immune system.

81. 600. FUNCTIONAL ASSESSMENT OF NOVEL STAT3 GENETIC DEFECTS IN ARGENTINEAN PATIENTS WITH INBORN ERRORS OF IMMUNITY

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Transcription factors, particularly STAT3, are essential in regulating the immune system by mediating responses to various cytokines. While many mutations affecting STAT3 have been reported, their precise molecular implications remain incompletely understood, necessitating further exploration. This study focused on elucidating the functional consequences of novel STAT3 mutations identified in Argentinean patients with Inborn Errors of Immunity (IEI). We examined eight novel missense STAT3 mutations, along with two mutations previously characterized as either gain-of-function (GOF) or loss-of-function (LOF). To assess the functional impact of these mutations, mutant STAT3 constructs were transfected into HEK293T cells. Subsequent experiments included activation and phosphorylation assays, and dimer formation studies were performed to evaluate mutant STAT3 activity and its interaction with wild-type (WT) STAT3. Among the mutations studied, p.Lys283Glu and p.Lys370Arg exhibited GOF activity similar to the well-documented p.Gln448Glu mutation, suggesting an enhanced ability to transduce cytokine signals. p.Leu598Phe, p.Pro669Ser, p.Leu673Pro, and p.Lys707Asn displayed LOF activity akin to the previously identified p.Phe621Leu mutation. This was evidenced by reduced activation compared to WT STAT3 following stimulation with IL-6, as measured by STAT3-driven GFP reporter expression. p.Phe384Tyr variant did not show differences from the WT. Phosphorylation assays revealed a spectrum of responses to IL-6 stimulation among the mutants, with deficits observed in those affecting the SH2 and trans-activation domains (p.Phe621Leu, p.Pro669Ser, p.Leu673Pro, and p.Lys707Asn). Additionally, mutations within these domains, specifically p.Leu673Pro, and p.Lys707Asn, impaired the formation of STAT3 dimers further compromising their functional capacity.
This study unveils novel STAT3 mutations in patients with IEI, underscoring the necessity for functional studies to validate their pathogenic potential and contribute to a deeper understanding of the molecular mechanisms underlying STAT3-related disorders. Such insights are vital for advancing personalized therapeutic approaches and improving patient outcomes.

82. 606. GROWTH OF PRIMARY IMMUNODEFICIENCY PATIENTS AT A NATIONAL PEDIATRIC REFERENCE CENTER IN PERU

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Background. Primary immunodeficiency diseases (PIDs) encompass a diverse group of genetic disorders that impact various components of the innate and adaptive immune systems. Despite their rarity, PIDs collectively pose a substantial disease burden, including growth retardation. However, there are few studies in growth and development in PID patients within Latin America. **Objective.** To assess the level of growth of PID patients at a national pediatric reference center in Peru. **Methods.** Cross-sectional study including pediatric PID patients at the Instituto Nacional de Salud del Niño during the study period (January 2023 and June 2024). The anthropometric measurements was compared in two groups, PID

patients and allergic diseases patients. Three anthropometric measurements were investigated: Weight-for-age, height- for-age and weight-for-height. **Results.** The study includes 62 PIDs patients (38 boys and 24 girls) and 30 allergic diseases patients. The age and sex distribution of both groups was similar. The height-for-age was 1.67z less in PID patients than allergic diseases patients (CI -2.5 to -0.8, p< 0.001), and The Weight-for- age was 1.5 z less in PID patients than allergic diseases patients (CI -2.5 to -0.58, p < 0.05). The height-for-age was 2.1 z less in Combined Immunodeficiency with syndromic features (CI -3.23 to -1.01, p< 0.001) **Conclusions.** Finally, the PID patients type II have lower height-for-age and weight-for-age. And the anthropometric measurements more committed in PID patients was the height-for-age

83. 626. IKZF1 DOMINANT NEGATIVE AND UNCOMMON IMMUNE-DYSREGULATION: CASE REPORT

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N159 *IKZF1* dominant negative causes a CID, myeloid defects and malignant predisposition. No evidence of immune-dysregulation associated up to date. Here we present a child with *IKZF1* DN disease who associates autoinflammation/autoimmunity conditions. A fourth son of non consanguineous and healthy parents with no familiar history of PID. At 10 months disseminated BCG infection (positive blood culture). At 12 months PCP pneumonia, untreatable oral thrush. Recurrent suppurative otitis and other viral respiratory and some gastrointestinal infections. Immunological investigations showed chronic neutropenia and lymphopenia (360-3000 cell/mm3). Pan agammaglobulinemia, lymphopenia CD19 (6-40 cell/mm3), lymphopenia CD3 (360 cell/mm3, CD4: 80 cell/mm3, CD8: 242 cell/mm3). Absent OKT3 proliferation *in vitro*. TH17 decreased. And a normal NK recount. IgRT, cotrimoxazole prophylaxis and r-metHuG-CSF were indicated. At 2 years old developed a Crohn's-like disease with extensive ulcers (chronic inactive colitis with apoptotic bodies). Thalidomide controlled ulcers but persistent diarrhea needed mesalazine (7 years old, partial response). At 6 yo, WES informed the known heterozygous ***IKZF1*** (c.476A>G p.Asn**159**Ser). Additional results: heterozygous ***NOD2*** (c.389C>T p.Pro130Leu) and heterozygous ***PIK3R1*** (c.2049G>T p.Glu683Asp) VUS variants. Familiar segregation: Father *NOD2/PIK3R1* carrier. Sister 1 *NOD2* carrier. Sister 2 *PIK3R1* carrier. Sister 3 (with AIH in adulthood) *NOD2* carrier. Altered NOD2 signaling upon stimulation with L18-MDP was evidenced in patient's neutrophils and monocytes by flow cytometry. PBMC showed decreased IGS of IL1β, IL8, IL6 and TNFα production when stimulated with MDP. At 8 years old he presented persistent hypertransaminasemia with positive anti-LKM antibodies (1/320) with later negativization. Viral infections and toxic conditions (without cotrimoxazol) were excluded. Pathological findings showed mild nonspecific lymphocytic infiltrate. Corticosteroids were initiated without response. Immune-dysregulation manifestations are not described to *IKZF1* DN and require the further discussion of *NOD2* and *PIK3R1* variants.

84. 635. CLINICAL PICTURE OF CHRONIC GRANULOMATOUS DISEASE? HOW ARE OUR PATIENTS DOING?

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Background: Chronic granulomatous disease

(CGD) is characterized by severe infections and inflammation. Management options include conservative treatment with prophylactic antimicrobial drugs or a curative approach through hematopoietic cell transplantation (HCT). Here we describe the clinical picture of the patients followed in our center. **Methods:** Retrospective data collection from 4 clinical records. 1 patient excluded due to lack of enough information. For patients with two HCTs, the analysis is based on the second procedure. **Results:** 3 male patients with X-linked CGD. Median follow-up is 7.3 years [2-10], median age at diagnosis is 12 years [1-31]. 2 patients underwent an HCT with a median age of 8 years [6-10]. One of the patients needed a second procedure with conditioning due to secondary graft failure. 1 patient is conservatively managed. Initially, patients had: 3 (100%) severe infections, 3 (100%) lung disease, 2 (66.6%) BCGitis, and 1 (33.3%) CGD colitis. Since the diagnosis, all (100%) received prophylaxis with Trimethoprim/Sulfamethoxazole, Antifungal agent (Itraconazole or Voriconazole) and Interferon-gamma. Patients with an HCT received peripheral blood stem cells (n=2) from matched (n=2) unrelated using both busulfan-fludarabine conditioning and serotherapy with Anti- thymocyte globulin. Cyclosporine and Methotrexate as GvHD prophylaxis. 1 patient (50%) developed acute skin GvHD but none of them had chronic GvHD. Both patients had CMV viremia needing treatment with valganciclovir. The patients that underwent HCT achieved a 100% donor myeloid chimerism with normal Dihydrorhodamine test, and no recurrence of CGD-symptoms. Post-HCT or with conservative management the 3 patients had no significant infections, no hospital admissions, improved or stabilized the lung disease and the one with CGD colitis resolved it. **Conclusions:** Long-term follow-up for CGD patients is mandatory, even after undergoing an HCT. Although our cohort is small either with curative or conservative treatment our patients showed significant improvement with both approaches.

85. 641. **COMPLEMENT ALTERATIONS IN A COHORT OF PEDIATRIC PATIENTS WITH POST-INFECTIOUS GLOMERULONEPHRITIS (PIGN)**

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Background: Post-streptococcal glomerulonephritis (PSGN) is the main cause of acute glomerulonephritis in pediatrics and develops after nephritogenic throat or skin infections. Although its clinical course usually resolves within weeks, few patients show an atypical form with persistent hypocomplementemia and kidney damage. **Aim:** To assess alterations in the complement system in children with PSGN. **Methods:** 11 patients with PSGN were enrolled. We measured C3/C4 levels by nephelometry, CH50/AH50 by hemolytic assays and C3Nef/PDC by functional assay. Atypical-persistent clinical course was characterized by an accelerated deterioration of renal function. **Results:** Patients (5 females, 6 males), median age: 9 years [2-15], median follow-up: 35.33±8.39 weeks [12-100], median ASTO: 866.8±180 UI/mL [165-2123], were all negative for antinuclear antibody test. 8/11 patients showed self-limited evolution and recovered within 8 weeks, median urinary protein:creatinine ratio (UPCR) was 2.17±0.53 mg/mg [0.5-12.16], transient C3 hypocomplementemia (median: 22.6±4.79 mg/dL), normal C4 levels (median: 18.4±1.75 mg/dL) and C3Nef transitionally positive (3/8 patients). Furthermore, they evidenced functional alteration in alternative complement pathway, with median AH50 activity of 46.4±6.9 minutes, and normal functionality of the classical pathway with median CH50 activity 59.7±8.1 U/mL. These patients showed a median normal estimated glomerular filtration rate (eGFR) of 75.54±6.44 ml/min/1.73m^2. 3/11 cases, all females, presented a torpid evolution after 6 months of follow-up, median UPCR was 8.65±2.87 mg/mg [5.13-12.16], C3 hypocomplementemia (median: 18.67±8.21 mg/dL), normal C4, with median AH50 activity greater than 60 minutes and C3Nef/PDC temporarily positive (all patients). Patients with atypical PSGN had an eGFR that decreased to 20 ml/min/1.73m^2. **Conclusions:** The patients with worst evolution showed functional time-persistent complement alterations according with constant low eGFR and higher proteinuria. These findings suggest that complement dysregulation could be linked to genetic alterations in the alternate path-

way.

86. 644. **MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASE, EXPERIENCE IN A PEDIATRIC REFERENCE CENTER IN ARGENTINA**

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Mendelian susceptibility to mycobacterial disease includes inborn errors of immunity with defects in the interferon-gamma and interleukin-12 pathway. The experience of a pediatric center in Argentina is reported. The clinical, microbiological and genetic-molecular findings were analyzed. Between 1996 and 2024, 28 patients from 24 non-consanguineous and unrelated families were diagnosed. There were 14 girls and 14 boys. The most frequent diagnosis was IL12RB1 deficiency (n=19), followed by partial IFNGR1 deficiency (n=4), STAT1 deficiency AD LOF (n=2), complete IFNGR1 deficiency (n=1), ISG15 deficiency (n=1) and IRF8 deficiency (n=1). Flow cytometry techniques (protein expression, post-stimulus pSTAT4 and pSTAT1 analysis) and genetic sequencing were used. 25 patients developed clinical manifestations with an average onset at 8 months old, and 23 of them had mycobacterial infections. 21 children present BCG infections after vaccination. 18 patients had disseminated infections, the most common sites were lymph nodes and lungs, and 5 had locoregional manifestations. In addition, coinfections were identified in 11 patients due to Salmonella (n=4), Candida (n=4) and Histoplasma (n=3). Different antibiotic regimens were used, using at least 2 drugs, for an average duration of 20±13 months. In addition, according to availability, 16 children received subcutaneous therapy with recombinant human interferon gamma-1b. 91.3% of patients achieved infectious remission, but 52.1% presented relapses due to mycobacterial infections, mainly BCG. Two children died, one with complete IFNGR1 deficiency due to disseminated BCG infection and another with IRF8 deficiency with the same infectious complication in addition to alveolar proteinosis.

To date, 3 patients remain asymptomatic (siblings of an index case, who did not receive the BCG vaccine). In Argentine pediatric patients, BCG is the main germ that causes infections in this group. Despite a good initial evolution, half of the patients relapse with new infections.

87. 649. **FOLLICULAR HELPER T CELLS: CLINICAL UTILITY IN INBORN ERRORS OF IMMUNITY**

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Introduction: Follicular helper T(Tfh) cells are subset of CD4+ T lymphocytes, required for the generation of germinal center, antibody producing and memory B cells. A small counterpart of Tfh named "circulating Tfh" (cTfh) can be measured in peripheral blood. They were classified into cTfh1/cTfh2/cTfh17 subsets. cTfh dysregulation may play an important role in inborn errors of immunity(IEI). **Aim:** 1)evaluate the clinical utility of cTfh compartment study and its subpopulations in different IEI. 2)Associate cTfh profiles with immunoglobulin G(IgG) levels, Bcell compartment and clinical features. **Materials and methods:**blood samples from 23 healthy donors (HD) and 29 patients (pts) with 18 different monogenic defects: were included. cTfh(CD45RA-CXCR5+), cTfh1(CXCR3+), cTfh17(CCR6+) and Bcell subsets(Naïve, Switched memory(Sw-MBL) and plasmablasts(PBC) were evaluated by flow cytometry. IgG was measured in serum by nephelometry. **Results**: cluster plot analysis of all mentioned parameters in our cohort could distinguish 3 different groups: G1= 8 pts, G2= 3 pts and G3=18 pts, of which G1 was the only group with homogeneous association of pts presenting as common variable immunodeficiency with autoimmunity and/or lymphoproliferation. Statistical analysis of cTfh frequencies of each group showed significant mean differences compared with HD only in G1 and G2 (11.6 vs 30.5 vs 31.4)($p<0.0001$). Only pts included in G1 group exhibited significant differences with HD in cTfh1 (mean 67.9 vs 30.3) and cTfh17 subsets (mean 7.0 vs 31.4)($p<0.0001$). cTfh1 correlated in the total cohort with Naïve($p<0.034$), Sw-MBL($p<0.0003$) and PBC($p<0.0024$). IgG levels correlated only with Sw-MBL ($p<0.026$). **Discussion**: cTfh measurement is not usually done in clinical laboratories. We show that an elevated cTfh percentage is not a marker of any specific genetic diagnosis. Specific mutations can dif-

ferentially affect the quantity and/or the quality of cTfh. Our findings evidence the utility of the cTfh1/cTfh17 ratio imbalance is useful to discriminate patients presenting with CVID phenotype.

88. 659. **IMMUNE COMPROMISE SECONDARY TO DENGUE INFECTION**

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INTRODUCTION: In daily clinical practice, it is often difficult to distinguish whether an immune compromise is triggered after an infectious process and/or immunosuppressive treatment or is a consequence itself or the onset of a primary immunodeficiency **OBJECTIVE**: To present a patient with a Common Variable Immunodeficiency (CVID) profile after treatment for refractory thrombocytopenia, secondary to Dengue infection. **METHODS:** Clinical Case Report **RESULTS:** In September 2021, a previously healthy 14-year-old female presented a dengue infection. Consequently, she developed thrombocytopenia (platelet: 33,000/mm3) with normal immunoglobulins levels. She received Immunoglobulin therapy 2g/kg, without response. so high-dose steroid was added, also with partial response. Therefore, she started rituximab treatment with 4 doses schedule, achieving normal platelet levels. After anti CD20 therapy she developed 2 pneumonias, an episode of bacterial conjunctivitis and generalized molluscum infection. The immunological assessment informed: Hypogammaglobulinemia with absent B lymphocytes and no functional response of protein and polysaccharide antibodies. Given her medical history and immune compromise, it was assumed as CVID. Nowadays, she is under treatment with weekly subcutaneous human immunoglobulin. **DISCUSSION:** Regarding this challenging case of a previously healthy woman whose developed CVID phenotype, it is difficult to define if it is a secondary immune compromise or the patient may present some molecular defect that predisposed her to present a viral infection that induced thrombocytopenia and subsequently lead to an immunological defect

89. 661. **NON-INFECTIOUS CHARACTERISTICS OF A POPULATION OF ADULT PATIENTS WITH INBORN ERRORS OF IMMUNITY AT HOSPITAL NACIONAL PROFESOR ALEJANDRO POSADAS**

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Introduction: Inborn errors of immunity (IEI) are a heterogeneous group of rare diseases caused by germline mutations in genes affecting the development or function of the innate or adaptive immune system. They are characterized by recurrent infections, autoimmunity (AI), allergies, and lymphoproliferation. **Objectives:** To characterize the population of patients with IEI treated in the Immunology Section. **Materials and Methods:** The medical records of patients with confirmed diagnosis of IEI, according to IUIS criteria, who were treated between 2015 and 2024, were analyzed to determine the demographic characteristics, autoimmune manifestations, and oncologic conditions of the study population. **Results:** A total of 355 adult patients with IEI were evaluated, 64% of whom were female. Of the diagnoses, 70% were classified as predominant antibody deficiencies. AI was present in 60% of the patients, with 76% of them being female. The most common autoimmune manifestations were hematologic (21%) and endocrinologic (18%). In 61% of cases, the AI diagnosis preceded the IEI diagnosis. All patients classified within groups IV and VII presented with AI. Oncological disease was diagnosed in 11% of patients, with 31% involving cutaneous manifestations and 22% with oncohematological manifestations. Only 28% of patients had a confirmatory genetic diagnosis. **Conclusion:** In adult patients with IEI, a predominance of females was observed, particularly among those with autoimmune manifestations, consistent with findings in the general population. In 61% of patients, the autoimmune disease preceded the diagnosis of IEI. Hematologic involvement was the most frequent autoimmune manifestation. Furthermore, a significant number of patients also presented with oncologic diseases. We conclude that adult patients with IEI exhibit characteristics that distinguish them from pediatric patients, highlighting the importance of proper characterization for improving diagnosis and treatment.

90. 664. **CLINICAL PICTURE OF MORE THAN 10 YEARS OF PATIENTS RECEIVING SUBCUTANEOUS IMMUNOGLOBULIN TREATMENT - SINGLE-CENTER EXPERIENCE**

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Background: Immunoglobulin administration is a mainstay in Inborn Errors of Immunity (IEI) with humoral defects. The FDA approved conventional subcutaneous immunoglobulin (SCIg) in 2006. It has been used in Argentina since 2010. Here we report the results of more than 10 years of this treatment. **Methods:** Retrospective (September 2010 – February 2024) data collection from 73 medical records. 61 patients (p) were included, 12 excluded due to lack of enough information. Study outcomes: 1. Efficacy based on: severe infections, need for hospital admission and IgG serum levels at 6 months; 2. Safety and Tolerability based on: adverse events (AEs) classified as local or systemic. Data analysis using descriptive statistics and percentages for qualitative variables. **Results: Study population:** 61p with a median age of 30.6 years [4 – 87]. 34 (55.7%) females, 27 (44.3%) males. 45 (73.8%) had pure humoral immunity involvement. Median follow-up of 5.1 years [0 – 12] receiving SCIg. **Treatment information:** 18 (29.5%) were naïve to treatment. Mean age of SCIg initiation 23.4 years [0 – 81]. 54 (88.5%) received replacement (<1gr/kg/dose) and 7 (11.5%) modulation dose (>=1gr/kg/dose). Most (32p, 52.5%) received weekly infusion and used one site (48p, 78.7%). **Efficacy:** Reduction from a median of 0.4 to 0.2 infections per year post-SCIg. Mostly upper or low respiratory tract, 15 (24.6%) needed hospitalization, no one in intensive care unit. Significant increase in serum IgG (>900mg/dl) after 6 months of treatment, from 21p (34.4%) to 42p (68.9%). **Safety and Tolerability:** 9p (14.8%) presented AEs, all local. 35 (57.4%) chose to continue with SCIg. 26p suspended due to: 8 (13.1%) medical indication, 4 (6.6%) patient decision, 4 (6.6%) switched to facilitated SCIg, 9 (14.8%) suspended follow-up and 1 (1.72%) passed away. **Conclusions:** Under SCIg the number of infections per year was reduced and this treatment was safe and well tolerated in patients with IEI.

91. 671. **FIRST STEPS WITH FACILITATED SUBCUTANEOUS IMMUNOGLOBULIN (FSCIG) THERAPY. A SINGLE CENTER EXPERIENCE**

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Introduction: Several studies have shown that facilitated subcutaneous immunoglobulin (fSCIg) is as good as intravenous (IVIg) and conventional subcutaneous immunoglobulin (cSCIg) preventing infections in innate errors of immunity (IEI). **Objective:** To describe the follow up of 21 patients with fSCIg treatment in the last 2 years in a single center in Argentina. **Methods:** Retrospective data collection from 21 clinical records (April 2022 – August 2024). **Results:** 21 patients (p) were included diagnosed with: 9p common variable immunodeficiency, 3p primary hypogammaglobulinemia, 3p specific antibody deficiency with normal Ig levels and normal B cells, 1p x-linked agammaglobulinemia, 1p hypogammaglobulinemia with IgG subclass deficiency, 1p specific antibody deficiency with IgA deficiency, 1p specific antibody deficiency and familial mediterranean fever, 1p LRBA deficiency, 1p autoimmune lymphoproliferative syndrome and IgA deficiency. Median age: 23.7 yo [0.2 - 67.7]. Mean time of follow up with fSCIg was 17.1 months [1.3 – 28.6]. Mean dose was 578.5 mg/kg/month [357 – 1000]. Mean serum IgG level was 1368 mg/dl [range 774 – 3130]. Annual rate of infection was 0,2 infections/year of follow up (2 bronchitis, 2 acute media otitis, 1 pneumonia, 1 giardiasis and 1 bilateral parotitis) and no one required hospital admissions. Tolerance: 7p (33%) reported local symptoms: 5p reported pain, swelling, erythema or pruritus that lasted less than 24 hours and 2 reported erythema and swelling of 4 and 5 days of duration respectively, no one of the local symptoms required treatment. 3 patients reported systemic adverse reactions (headache and/or fever) only during the first infusions. **Conclusion:** fSCIg therapy is safe and effective for replacement treatment in patients with IEI. Further systemic clinical studies are needed to better define optimal dosage and application intervals.

92. 672. **AN UNCLASSIFIED IMMUNODEFICIENCY**

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Introduction: Daily work in clinical immunology often involves the follow-up of patients with immunocompromised conditions without being able to classify them under a specific immunodeficiency or whose clinical diagnosis may change over time. **Objective:** To describe a case of an unclassified immunodeficiency. **Methods:** Case report. **Results:** 40 years old female with unremarkable family history who has had recurrent suppurative otitis since childhood requiring several surgeries for cholesteatomas that lead to left tympanic perforation and secondary bilateral hearing loss. Clinical picture also showed: Recurrent pharyngitis, labial herpes, oral canker sores, arthralgias, intermittent chronic diarrhea and leukopenia. In 2020 mild hypogammaglobulinemia, no response to polysaccharide vaccine, slightly low B and NK cells were detected. Thymomodulin and azithromycin prophylaxis were indicated without improvement, adding immunoglobulin replacement treatment in 2021. Sequence and Del/Dup (CNV) analysis using the Blueprint Genetics (BpG) Primary Immunodeficiency Panel of 298 genes in 2020 did not detect any known disease-causing or rare variants. Since April 2022, she has reported night sweats, chills and lower limbs oedema, without fever. She has a normal venous Doppler ultrasound of the lower limbs, negative sputum culture. Since she started treatment with immunoglobulin replacement she did not suffer from new respiratory infections, but persisted qith isolated otitis (<1 episode/year) and few episodes of diarrhea and abdominal distension. In May 2024 she had otitis recurrence so azithromycin prophylaxis was initiated achieving a free infection period. **Discussion:** Since 2020, there have been described several new autoinflammatory disorders and diseases of immune dysregulation. Therefore, it was requested to extend the genetic test. Although the infections decreased with the immunoglobulin replacement treatment, it is difficult to define if the night sweats and edema could be secondary to the treatment.

93. 675. **LYMPHOCYTIC GRANULOMATOUS LIVER DISEASE IN XIAP DEFICIENCY: CASE REPORT**

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Introduction: XIAP deficiency causes hemophagocytic lymphohistiocytosis (HLH), inflammation and susceptibility to Epstein-Barr virus (EBV) in men. Liver involvement is rarely reported outside of HLH and EBV infection. Of eleven patients diagnosed in our service, one patient presented immune-mediated liver involvement. **Case presentation:** First child of healthy non-consanguineous parents. First episode of HLH at 2 months of age, subsequently recurrent HLH not associated with EBV. At 13 months, diagnosis of XIAP defect (NKT 0.01%, decreased XIAP protein expression, c.1056+3_6 variant of 4bp in intron 4 of the *BIRC4* gene). Immunoglobulin replacement therapy was started. At 6 years, increased transaminases were identified, without clinical manifestations with normal ultrasound. FAN, ASMA and ANTI-LKM antibodies were negative. Only fluctuating herpes 6 viremia was documented. Liver biopsy showed mild fibrosis, CD4 T lymphocyte infiltrate with histiocytosis forming microgranulomas. HV6 PCR, CMV PCR and *in situ* hybridization for EBV negative. It was interpreted as immune-mediated hepatitis, and treatment with methylprednisolone (0.5 mg/kg/day) and azathioprine (0.5 mg/kg/day) was started. After fifteen days, transaminases normalized. Currently, after two years and four months, the patient is in total remission with low-dose corticosteroids and azathioprine, with good tolerance. **Conclusion:** Immune-mediated hepatitis could be part of the clinical spectrum of XIAP deregulation. First- instance immunosuppression was effective in our case.

94. 676. **CHRONIC GRANULOMATOUS DISEASE IN AN ADULT FAMILY**

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Background: Chronic granulomatous disease (CGD) is a primary immunodeficiency disorder of phagocytes resulting from impaired killing of bacteria and fungi. It may present anytime from

infancy to late adulthood; however, the vast majority of affected individuals are diagnosed during early childhood. **Objective:** To describe an adult family with late diagnosis of CGD **Methods:** Clinical information was retrieved from medical records. **Results:** Our index patient is a 31 year old previously healthy man, who was admitted to the hospital with headache and generalized seizures secondary to a right temporal nodule. Additionally, a nodular lung lesion was found. With all cultures negative, a lung biopsy was made demonstrating noncaseating granulomas with giant cells. Fungal cultures were positive for a dematiaceous fungus called Cladophialophora bantanii. HIV and tuberculosis tests were negative. There was no evidence of autoimmunity or oncologic disease, and he had normal immunoglobulin levels and lymphocytes subsets. He has a brother (35 y.o) who had BCGitis in infancy and at 15 he developed axillary adenopathy with granulomas diagnosed as sarcoidosis. In his twenties, he had prolonged fever with adenopathies. Their mother (76 y.o) was diagnosed with Discoid Lupus and Rheumatoid arthritis. They underwent a dihydrorhodamine burst (DHR) assay which revealed low enzyme activity (2.67 % normal oxidative burst) in both brothers and the mother as a carrier. Genomic analysis revealed a pathogenic variant in CYBB gene, exon 9 (p.Glu309Lys). Both brothers received prophylaxis with itraconazole, trimethoprim-sulfamethoxazole and interferon gamma. **Conclusion:** The originally described "fatal granulomatous disease of childhood" has become an adulthood chronic disease. Most missense variants in CYBB are associated with higher residual activity, which may explain late onset of the disease. However, life threatening complications may occur and a curative treatment like haemopoietic cells transplant should be considered.

95. 678. **CHRONIC GRANULOMATOUS DISEASE IN FEMALE CARRIERS**

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Background: Chronic granulomatous disease (CGD) is a hereditary immunologic disorder with impaired activity of phagocyte NADPH-oxidase. X-Linked CGD results from mutations in the CYBB gene and female carriers have a dual phagocyte population due to lyonization. They may develop symptoms of intestinal bowel disease (IBD), autoimmunity and severe infections. *Objectives:* To describe clinical manifestations in 4 CGD-carriers women. *Methods:* Clinical information was retrieved from medical records. *Results:*S1 (70 y.o.) suffers from double-positive Rheumatoid Arthritis (RA) refractory to cDMARDs and anti-TNF-therapy. She also has sicca syndrome and atopic dermatitis, with high titles of homogeneous ANA, anti-DNA antibodies and hypocomplementemia. She has 47% normal oxidative burst and a pathogenic missense variation in CYBB, exon 9 (c.925G>A). Her daughter (36 y.o), S2, presents intermittent arthralgias, sicca syndrome, mild acne and non-specific gastrointestinal symptoms with negative autoantibodies. S3 (76 y.o) has Discoid Lupus and seropositive RA. She has 43% normal oxidative burst and a missense variation in CYBB, exon 9. S4 (36 y.o) suffers from seronegative arthralgias and pheochromocytoma with a pathogenic mutation in the SDHB gene, which is related to hereditary paraganglioma-pheochromocytoma syndromes. We report 4 female carriers (S1-S4): all of them with autoimmune and inflammatory manifestations: half with highly positive autoantibodies and most with skin and gastrointestinal symptoms. *Discussion:* CGD-carriers have a wide-ranging inflammatory and autoimmune manifestations, usually seronegative Lupus-like syndromes and IBD, with poor correlation with DHR levels. Our cohort had subjects with high autoantibody levels, some of them refractory to standard therapies. All variants were missense, which are suggested to have more superoxide production, probably explaining the absence of severe infections.
Identifying carriers most at risk of developing symptoms is challenging because there are no clear predictor markers. XL-CGD carriers must be managed proactively.

96. 684. **HETEROZYGOUS TCF3 MUTATION IN A PATIENT WITH AGAMMAGLOBULINEMIA**

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INTRODUCTION: TCF3 is a transcription factor

that plays a key role in early lymphocyte differentiation. Germline monoallelic dominant negative and biallelic loss-of-function (LOF) null TCF3 mutations cause a fully penetrant severe immunodeficiency. **AIMS:** To describe the clinical presentation of a patient with heterozygous mutation in the TCF3 gene **METHODS:** A retrospective review was conducted on the clinical history of a 5-year-old female patient. **RESULTS:** The patient, 5y.o. girl presented with syndromic features, including hypertelorism, low-seat ear and telangiectasias on her cheeks, with normal karyotype. At the age of 3y.o, she experienced her first severe infection, requiring hospitalization due to bilateral necrotizing pneumonia, which resulted in neurological sequelae. She also developed DRESS syndrome secondary to treatment. Immunological studies revealed a significantly decreased levels of IgG, IgA and IgM, along with chronic absent B lymphocytes. Given the severity of her infection and her abnormal immunological profile characterized by panhypogammaglobulinemia, gammaglobulin replacement therapy was started, resulting in adequate plasma IgG levels and no subsequent infections. Genetic testing identified a novo pathogenic variant was in the TCF3 gene (c.1663G>A; p.E555K) **DISCUSSION:** The identified variant in the TCF3 gene is located in a critical functional domain of the protein, likely contributing to the patient's phenotype, including agammaglobulinemia and infection. This study highlights the importance of genetic analysis in clarifying the molecular basis of complex clinical presentations and emphasizes the need for personalized management strategies **Conclusions:** Our study identifies a mutation hotspot in the TCF3 DN variant and underscores the weak negative selection associated with the TCF3 gene, shedding light on its role in immunodeficiency

97. 697. **ATYPICAL CLINICAL PHENOTYPE OF FAMILIAL MEDITERRANEAN FEVER**

Agostina Llarens[1], Lucía Peirano[1], Matías García[1], Graciela Betancur[1], Sabrina Argüello[1], Tatiana Velasco[1], Mayra Echeverry[1], María Julia Papagno[1], Alejandra Abalo[1], Soledad Martinez[1], Adrián Estevez[1]

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Background: Familial Mediterranean fever (FMF) is a monogenic autoinflammatory disease. Common symptoms include: self-limited inflammatory episodes of fever and polyserositis and high acute phase response. Other organ impacts are uncommon. **Objectives**: To report an atypical clinical case of FMF **Methods**: Retrospective clinical records analysis. **Results**. She is a 21-year-old (yo) woman born from Syrian origin non-consanguineous parents. She has a history of adrenal insufficiency at 5yo, chronic splenomegaly and hepatitis with portal hypertension at 16yo. Additionally, she developed intermittent cytopenias with normal bone marrow aspiration. During the last years, she suffered from recurrent abdominal pain, initially assumed secondary to gastritis. However, abdominal pain persisted associated with a periodic tender added to a purpuric low limbs rash and erysipela-like lesions. An autoinflammatory disease was suspected. A genetic test revealed a homozygous variant in *MEFV* p.Met694Val in the patient and both symptomatic sisters. Her parents are heterozygous carriers. Since the diagnosis, she received colchicine 0,5 to 1mg/day (for 3 years) with regular tolerance. She manifested an improvement in abdominal pain but not completely solved. It was decided to start Canakinumab 150mg/month. Unfortunately, after 3 months of treatment, she persisted with some FMF crisis. She reached to control the disease with a double dose of Canakinumab. However, she requires hydrocortisone and a strict hepatology follow-up. **Conclusion**. M694V *MEFV* homozygous variation is associated with severe clinical phenotype. The liver is usually intact. When it is involved, the damage could be mild or develop a cryptogenic cirrhosis. Intermittent cytopenias are not typically described. Here, cytopenias improved with Canakinumab, therefore they were assumed secondary to the inflammatory crisis. Even though fever in FMF is present in 93% of the patients, it is not mandatory. There is no clear data on the prevalence of adrenal involvement in patients with FMF.

98. 703. **ADA 2: A SYMPTOMATIC HETEROZYGOUS PATIENT. CASE REPORT.**

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BACKGROUND: Deficiency of Adenosine deaminase 2(DADA2) is an inborn error of immunity-IEI presenting a broad spectrum of clinical manifestations, including immunodeficiency, vasculopa-

thy, and hematologic disease. Biallelic mutations in the ADA2-gene have been associated with decreased ADA2-activity. Heterozygotes(carriers) are asymptomatic and are not thought to be at risk of developing the disorder. **OBJECTIVE:** To present a 35-year-old woman with hematologic phenotype and heterozygous pathogenic variant in the ADA2-gene. **METHODS:** A 35-year-old woman presented with intermittent fever, neutropenia, anemia and livedo reticularis. Family without history of IEI and non-consanguineous parents. Personal records: preterm newborn product of a twin pregnancy. No relevant issues during her infancy. At 27 she presented lymphoproliferation and fever, which was assumed as Mononucleosis syndrome. At 28yo she suffered from a miscarriage at 6 weeks. After that, she started with intermittent fever, neutropenia, and anemia. At 33yo, she got a SarsCOV-2 infection complicated with permanent loss of smell with damage to the olfactory tract seen in RMI. Neutropenia and megaloblastic anemia were revealed. Bone marrow showed hypoplasia of red and myeloid cells with increased CD8 T-Cells. Infections, metabolic, rheumatological, and hematological diseases were ruled out. Complementary studies:CTbodyscan, abdominal ultrasounds, and PETbodyscan were normal. Immunological workup: Immunoglobulins were in range and antibody responses to vaccines were normal. Epstein Barr and CMV IgG were positive. Flow cytometry assays showed: CD8+T cells-effector increased, NK was almost absent, CD19+ decreased and CD21low increased. An IEI was suspected. Molecular extended panel>500 genes showed a heterozygous missense pathogenic variant in ADA2 c.1078A>G-p.Thr360Ala. Measurement of plasma ADA2 enzymatic activity was in the carrier range. **DISCUSSION:** Importantly, whether heterozygous pathogenic variants predispose to DADA2 manifestations remains to be investigated.

99. 704. CLINICAL SPECTRUM OF SELECTIVE IMMUNOGLOBULIN A DEFICIENCY IN A SINGLE CENTER

Lucia Peirano (Centro de Inmunologia Bezrodnik), Gustavo Marin (Centro de Inmunologia Bezrodnik), Gisela Seminario (Centro de Inmunologia Bezrodnik), Pilar Tejada (Centro de Inmunologia Bezrodnik), Ileana Moreira (Centro de Inmunologia Bezrodnik), Agostina Llarens (Centro de Inmunologia Bezrodnik), Matias Garcia (Centro de Inmunologia Bezrodnik), Liliana Bezrodnik (Centro de Inmunologia Bezrodnik) **Background:** Selective immunoglobulin A deficiency (SIgAD) is the most common inborn error of immunity. Most of the patients are asymptomatic, others suffer different clinical complications such as pulmonary infections, allergies, autoimmune diseases, gastrointestinal disorders and malignancy. **Objectives:** To describe the wide clinical spectrum of eleven patients(pt) with symptomatic SIgAD in a single center in Argentina. **Methods:** Retrospective data was collected from clinical records. All patients were diagnosed with SigAD according to guidelines. **Results:** 11-pt with a diagnosis of SigAD. Sex distribution: 27% females and 73% males. Overall median-age was 23 years[7-75]. Clinical manifestations included: recurrent sinopulmonary infections 37%, autoimmunity 36%, autoinflammatory 18%, and oncohematologic disease 9%. Within the autoimmunity phenomena: autoimmune cytopenias, celiac disease, vitiligo, Hashimoto's thyroiditis, and type I diabetes were presented. Three patients present lymphoproliferation, two of them met the criteria for Autoimmune lymphoproliferative syndrome(ALPS) and ALPS-like. Treatment: Five pt are under IgG replacement therapy, four due to severe infection, and another as immunomodulatory therapy. Only one is under antibiotic prophylaxis. Regarding, immunological assessment, concomitant to SigAD 4/11 present increased IgE levels (>100 IU/ml, IQR:836 IU/ml). All had normal antibody responses to tetanus toxoid and 36% presented abnormal antibody responses to Pneumococcal polysaccharide. A decrease in the percentage of switched memory B-cell subsets was observed in 45 % and an increase in the percentage of CD-21low B-cells was observed in 60% of the cohort, these patients were associated with severe phenotypes (recurrent and intensive infection and autoimmunity. **Conclusion:** In our cohort, as has been already reported, the patients with more severe clinical manifestations have presented with lower switched memory B-cells and increased cd21low B-cells.

100. 716. RECURRENT CAMPYLOBACTER BACTEREMIA IN PATIENTS WITH ANTIBODY DEFICIENCY

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1. Hospital C.G.Durand

Background: Campylobacter infection, usually Campylobacter jejuni, is the most common cause

of bacterial enteritis worldwide. In healthy individuals, the infection is self-limiting, but in immunodeficiency patients recurrent or persistent infection may occur. **Objectives:** To describe a patient's serie with antibody deficiency who suffered recurrent Campylobacter bacteremia, highlighting the relevance of the diagnostic suspicion of this underreported complication. **Methods:** The medical records of three patients with a diagnosis of antibody deficiency and recurrent Campylobacter bacteremia. **Results:** Case 1: 64-year-old male with hypogammaglobulinemia (secondary vs. primary due to the finding of a variant in FASLG), with a history of Primary Sclerosing Cholangitis (PSC) and NHL in remission, under immunoglobulin replacement therapy (IgRT). During follow-up the patient presented fever with isolation of C. coli in blood cultures (BC), with recurrence at 1 month and 3 months, despite antibiotic treatment. Case 2: 73-year-old female with secondary hypogammaglobulinemia, on IgRT and a history of PSC, CLL, gastric adenoma with intestinal metaplasia and multiple primary solid tumors. She underwent studies for prolonged fever and C. coli grew in BC; with recurrence at 2 month and 3 months, despite antibiotic treatment. Case 3: 27-year-old female with common variable immunodeficiency due to NFkB1 deficiency under irregular IgRT. Subtotal gastrectomy for severe gastric atrophy with low-grade dysplasia. Chronic diarrhea was interpreted as inflammatory bowel disease-like. She was hospitalized for fever and diarrhea with C. jejuni on BC + positive PCR in feces; with recurrence 2 months later, despite antibiotic treatment. **Conclusions:** Recurrent Campylobacter infection can contribute to significant morbidity in patients with antibody deficiency. A high index of suspicion should be maintained in these patients, considering that it is difficult to isolate even in specific media. There are still no treatment guidelines for this complication in patients with immunodeficiency

101. 717. **FLOW CYTOMETRY FINDINGS IN ACTIVATED PI3KΔ SYNDROME PATIENTS**

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1. Hospital C.G.Durand

Background: activated PI3Kδ syndrome (APDS) is characterized by recurrent respiratory tract infections, lymphoproliferation, increased lymphoma susceptibility, and poor antibody production. It may present as common variable immunodeficiency or hyper-IgM syndrome. Increased transitional B cells is a common finding but flow cytometry phenotypes may show heterogeneous findings. Objectives: describe flow cytometry phenotypes in patients with molecular diagnosis of APDS 1 or 2 and correlate with clinical manifestations Materials and methods: we analyzed patients >18 years old with mutations in PIK3CD or PIK3R1 who were assessed at the Immunology Unit of Hospital C.G.Durand and who had flow cytometry immunophenotype performed at our laboratory. Results: we analyzed 6 patients from 4 unrelated families, 5/ 6 were females. Four of six patients had heterozygous variants in PIK-3CD and two in PIK3R1. Four of them presented as hyper-IgM syndrome, one patient as agammaglobulinemia and one had normal immunoglobulin levels. One patient had CD19 0%, decreased IgA, IgG and IgM levels, he developed fulminant lymphoma years later. One patient with prominent lymphoproliferation had very low B cells, absent IgA levels, increased CD21low B cells and a remarkably increased count of NK cells Two patients (from the same family) had very increased plasmablasts and decreased naive and memory B cells, both with recurrent sinopulmonary infections, hepatosplenomegaly and chronic diarrhea. Their mother had normal immunoglobulin levels and mild B cell lymphopenia with occasional respiratory infections One patient with bronchiectasis and lupus nephritis had increased IgG and IgM levels, decreased naive B cells and normal transitional and memory B cells Conclusions: there is a wide spectrum of flow cytometry findings that may be related to different clinical features. Further analysis is needed to clarify the heterogenous manifestations of APDS

102. 719. **LATE DIAGNOSIS AND SEVERE AUTOIMMUNE MANIFESTATIONS IN A PATIENT WITH PI3K-DELTA ACTIVATED SYNDROME (APDS-1): A CASE REPORT**

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Background: The PI3K-delta Activated Syndrome (APDS) is a primary immunodeficiency caused by a heterozygous variant with gain of

function in the PI3Kδ gene, either in its catalytic site (APDS-1), or regulatory site (APDS-2), resulting in hyperactivation of the PI3K cellular signaling pathways, generating a wide spectrum of clinical manifestations. **Objective**: To describe a case of APDS-1 in a patient with late diagnosis and severe autoimmune manifestations, and to discuss treatment. **Method:** We revised the case of a patient with APDS-1 and her follow-up in our center. **Results**: A 38 year-old female patient with APDS-1, who has suffered recurrent pneumonias since 5-year-old. At 33-year-old she started follow-up at our center. Laboratory results showed hypogammaglobulinemia and lymphopenia. She have a daughter with Common variable immunodeficiency, with subsequent identification of heterozygous variant p.Glu1021Lys in exon 23 of the PIK3CD gene, also found in our patient later. When she was aged 36 she was hospitalized for ascitic syndrome, renal impairment, proteinuria >4gr; positive antinuclear and anti DNA-antibodies. Lupus Nephritis Class IV was diagnosed by biopsy. She received treatment with cyclophosphamide and mycophenolate. At 37 years old, she presented varicella-zoster reactivation with dorsal and oral lesions, treated with acyclovir with persistence of oral and pharyngeal lesions. Cytomegalovirus was isolated in the lesion swab. Immunosuppression was discontinued and began Ganciclovir with response. During the same year, she had worsening of tongue lesions when resuming immunosuppression, with swab positive for Candida. Currently, with improvement of lupus activity while maintaining treatment with Mycophenolate; her infectious intercurrences force interruptions. **Conclusions**: APDS represents a challenge not only for diagnosis due to the breadth of its clinical manifestations. We also discuss whether targeted therapies or transplantation would be better options than actual treatment for our patient

103. 728. **COMPLEX PHENOTYPE: CRANIOFACIAL AND SKELETAL ANOMALIES, LYMPHOPROLIFERATION AND HYPOGAMMAGLOBULINEMIA**

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Background: Predominant antibody deficiencies can manifest within various syndromic phenotypes. However, the combined contribution of genetic variants in patients with suspected Inborn Errors of Immunity and syndromic features remains poorly understood. **Hypothesis:** Patients with complex clinical features could show more than one genetic variant combined to explain the whole phenotype. **Objectives:** This study aimed to describe the genetic profile of a term-born boy without significant family history, presenting a complex phenotype characterized by craniofacial and skeletal anomalies, lymphoproliferation, and hypogammaglobulinemia. **Method:** We revised a patient's case and his exome through Whole exome sequencing (WES). **Result:** The patient exhibited failure to thrive, developmental delay, distinctive facial features, and skeletal abnormalities, along with recurrent pneumonia, chronic pulmonary disease, B-cell lymphopenia WITH hypogammaglobulinemia, mild CD4 T-cell lymphopenia and Hodgkin lymphoma at 12 y.o., treated with radio and chemotherapy with complete remission. Comprehensive clinical, immunologic, and genetic analyses were conducted to elucidate the complex phenotype. WES identified a homozygous variant in TNFRSF13B (c.310T>C, p.Cys104Arg), classified as likely pathogenic, commonly associated with common variable immunodeficiency and lymphoproliferation. Additionally, a heterozygous variant in NOTCH2 (c.4457C>T, p.Thr1486Met) was detected, categorized as a variant of uncertain significance with extremely low frequency in GnomAD 4.0 (13/1,614,180 alleles), with a Revel score of 0.75, potentially explaining craniofacial, skeletal, and neurologic features. **Conclusions:** Both TNFRSF13B and NOTCH2 variants may contribute to the observed complex phenotype, providing insights beyond predominant antibody deficiencies with syndromic features and highlighting the importance of comprehensive genetic evaluation in such cases.

104. 038. **PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA COACTIVATOR 1-ALPHA AT THE INTERFACE BETWEEN MITOCHONDRIAL ACTIVITY AND REGULATORY T CELL BIOLOGY**

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Regulatory T (T_{reg}) cells play an important role in the maintenance of immunologic tolerance and control of immune responses. In the last few years, it has been evidenced different metabolic pathways driving the phenotype of T_{reg} cells, which require mitochondrial integrity and metabolism to exert their functions. Mitochondria are sites of biochemical processes, including lipid oxidation, tricarboxylic acid cycle, and oxidative phosphorylation, that culminate in the generation of energy. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1a) acts as an important regulator of oxidative metabolism, biogenesis, and mitochondrial dynamics through the activation of different transcription factors, being widely described in tissues with high energy demand. Although a low expression of PGC1a has been related to the exacerbation of inflammatory responses, little is known about its intrinsic role in immune cells, especially in immunoregulatory functions. This work aims to investigate how PGC1a connects the regulation of cellular metabolism to the generation and function of T_{reg} cells. For that, we performed murine and human in vitro cultures of iTreg cells in the presence/absence of PGC1a modulators and evaluated cell differentiation and mitochondrial parameters. Our results demonstrated that higher mitochondrial content is closely related to the differentiation of T_{reg} cells. Moreover, these cells showed a substantial increase in the expression of PGC1a. In line, increased PGC1a activity promoted higher levels of Foxp3 expression, which effect was reduced by the inhibition of PGC1a, suggesting a contribution of PGC1a in the development of T_{reg} cells. Also, the activation of PGC1a was accompanied by increased mitochondrial mass and mtDNA levels in Treg cell cultures. Moreover, we also observed that PGC1a plays a role in the differentiation of human Treg cells in vitro. These preliminary findings shed light on the PGC1a potential as a pharmacological target when manipulating Treg cells as a therapeutic strategy.

105. 044. HIF2-ALPHA ACTIVITY IN MYELOID LEUKOCYTES PROMOTES SUSCEPTIBILITY TO MYCOBACTERIUM TUBERCULOSIS INFECTION

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Human tuberculosis (TB) is caused by the bacillus *Mycobacterium tuberculosis* (Mtb). TB has a high annual mortality rate. Mtb primarily resides in macrophages and orchestrates metabolic alterations to destructing and amplifying its replication. Host-directed therapies have been explored as new treatment alternatives. The hypoxia-inducible factor (HIF) family of transcription factors influence the metabolism and activation of leukocytes. HIF1α-mediated polarization of M1 macrophages is crucial for host defense against Mtb infection. In contrast, HIF2α is known to induce M2 macrophages polarization, but its importance in host defense against TB remains unclear. Then the objective is to determine whether HIF2α signaling in infected host myeloid cells plays a harmful role. We infected C57BL/6 mice with 100 or 1000 colony-forming units (CFU) of Mtb and 4 weeks post-infection (spi). The lung homogenate showed lower HIF2α mRNA expression compared to uninfected animals. However, when we quantified HIF2α expression in the lungs by flow cytometry. Among infected lung myeloid cells, HIF2α was mainly detected in macrophages. In vitro, we observed pharmacological inhibition or genetic deletion of HIF2α resulted in greater resistance to infection. The increased resistance of HIF2α KO macrophages to Mtb infection was not associated with iNOS expression or NO production, but HIF1α gene and protein expression levels were higher in HIF2α KO macrophages compared to wild-type cells (WT) infected by Mtb. In vivo, treatment with a pharmacological inhibitor of HIF2α (PT-2385) reduced lung bacterial levels in Mtb-infected C57BL/6 mice, in addition HIF2α KO mice were also more resistant to Mtb infection compared with WT mice. We found higher expression of HIF1α in the lungs of conditional HIF2α KO mice compared to WT animals. Our data suggest HIF2α in myeloid leukocytes promotes susceptibility to Mtb infection, potentially

indicating a protective role arising from HIF1α expression in macrophages when HIF2α is deleted.

106. 045. **NEUTROPHILS MITOCHONDRIAL RESPIRATORY COMPLEXES ARE DIFFERENTIALLY ARRAYED BY EXPOSURE TO METABOLITES AND THIS IS RELATED TO CHANGES IN THEIR MICROBICIDAL ACTIVITY ON STAPHYLOCOCCUS AUREUS**

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Neutrophils are regarded as the first line of defense against a wide range of pathogens. Several aspects of neutrophil function still remain unclear; in this regard, the role of mitochondria in neutrophils function has recently gained attention. We previously observed that metabolites such as lactate (L), succinate (S), fumarate (F), acetate (A) and butyrate (B) induce differential mitochondrial reprogramming in monocytes. Here, we aimed to analyze the impact that the above-mentioned metabolites have on the mitochondrial respiratory complexes and the possible relationship with their microbicidal activity and NETs release. Purified human blood neutrophils were exposed separately to L, S, F, A or B at 100 µM final concentration, followed by 4 hours stimulation with PMA. Neutrophils mitochondria were isolated, and the array of mitochondrial respiratory complexes was analyzed by anti-total OXPHOS Western blots. Neutrophils exposed to the same metabolite conditions were challenged with *S. aureus* and their microbicidal activity was assessed by UFCs. Results showed that exposure of neutrophils to the different metabolites leads to a different array of mitochondrial complexes (I, II, III, IV and V), and differential microbicidal activity against *S. aureus.* Likewise, different arrays of mitochondrial complexes were observed in the course of PMA-induced NETs release. We propose that the metabolic microenvironment of neutrophils determines the abundance of mitochondrial individual respiratory complexes, and that this may be related with the differential neutrophils microbicidal and NETs release capacity.

107. 072. **EXAMINING THE RELATIONSHIP BETWEEN IL-10, APOPTOSIS, AND AUTOPHAGY IN MASLD AND OBESITY**

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Metabolic dysfunction-associated steatotic liver disease (MASLD) encompasses a spectrum of liver conditions, from simple steatosis (MASL) to steatohepatitis (MASH), frequently associated with obesity. The development and progression of MASLD involve fundamental cellular mechanisms and their corresponding genes: 1) endoplasmic reticulum stress (*PDIA3*, *CHOP*, *PERK*); 2) oxidative stress (*NOX*, *PRDX6*); 3) autophagy (*ATG3*, *ATG12*, *LC3B*); 4) inflammation (*IL6*, *TNF*, *IL1B*); and 5) apoptosis (*TRAILR2*, *BAX*). Anti-inflammatory cytokines, like IL-10, are critical in mitigating hyperactive immune responses and preventing hepatic cellular damage. Our research demonstrated that serum and hepatic IL-10 levels in obese patients are correlated with lobular inflammation, a precursor to MASH.

To elucidate the role of IL-10 in MASLD, we explored the relationship between serum and hepatic IL-10 levels and the expression of MASLD-associated genes (*PDIA3*, *CHOP*, *PERK*, *NOX*, *PRDX6*, *ATG3*, *ATG12*, *LC3B*, *IL6*, *TNF*, *IL1B*, *TRAILR2*, *BAX*) in individuals with obesity and MASLD. IL-10 levels were quantified via ELISA in serum and liver samples from 39 patients with obesity and MASLD, while hepatic gene expression was assessed through RT-qPCR. Pearson correlation coefficients were calculated to examine the associations between IL-10 levels and hepatic gene expression, with statistical significance $P<0.05$. We found a strong positive correlation between serum and hepatic IL-10 levels ($r=0.6466$, $P<0.001$), indicating synchronized regulation of this cytokine systemically and within the liver. Additionally, a significant negative correlation was observed between serum IL-10 and the pro-apoptotic gene *BAX* in the liver ($r=-0.5091$, $P=0.0011$) and between hepatic IL-10 and *BAX* ($r=-0.3089$, $P=0.0380$). Furthermore, mild positive correlations were identified between IL-10 and *ATG3* in serum ($r=0.3340$, $P=0.0331$) and liver ($r=0.3068$, $P=0.0466$). Our findings suggest

that IL-10 is intricately related to hepatic apoptosis and autophagy, two critical mechanisms in MASLD pathogenesis. This discovery opens exciting possibilities, as IL-10 could serve as a prognostic biomarker and a therapeutic target for this disease.

108. 075. **IS THE TH2 IMMUNE RESPONSE THE UNDERLYING MECHANISM BETWEEN METABOLIC-ASSOCIATED STEATOTIC LIVER DISEASE AND THE SYSTEMIC ARTERIAL HYPERTENSION?**

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Non-alcoholic fatty liver disease (NAFLD), now known as metabolic-associated steatotic liver disease (MASLD), is alarmingly on the rise alongside the global increase in obesity cases. MASLD is an underestimated metabolic abnormality that is closely linked to an increased risk of developing systemic arterial hypertension (SAH). Although clinical studies have shown a reciprocal relationship between these two conditions—i.e., SAH is identified as an independent predictor of MASLD, and in turn, MASLD is associated with a higher risk of developing SAH—the underlying mechanism of the association between MASLD and SAH remains unknown. Inflammation may be a link between these two conditions, as during MASLD, Kupffer cells synthesize and release pro-inflammatory cytokines into circulation, causing a state of chronic low-grade inflammation that can lead to dysregulation of critical pathways involved in blood pressure regulation, such as the renin- angiotensin system (RAS). Previous studies have shown that pharmacological inhibition of ACE (a key molecule in the classical RAS pathway) affects interleukins 4 and 10, which are effector cytokines of the Th2 immune response. In this study, we determined in liver biopsies from individuals with morbid obesity and MASLD a strong correlation of cytokines IL-4(r=0.598, *P*<0.0001), IL-10 (r=0.516, *P*<0.0001), and IL-13 (r=0.671 *P*<0.0001) with ACE protein expression. Furthermore, through multiple linear regression analysis, IL-4 and IL-13 were found to be the best predictors of ACE levels in these liver biopsies (r^2=0.3898, *P*= 0.0002). Interestingly, we also found an overexpression of IL-13 in patients with morbid obesity, MASLD, and SAH (9.45±4.25pg/mL) compared to those without SAH (7.25±4.44pg/mL). These findings lead us to propose, for the first time, that the Th2 response, through the regulation of the RAS pathway, could play a critical role in the development of SAH in individuals with MASLD. The results of this project reveal molecular mechanisms in the MASLD-SAH relationship, specifically the likely involvement of the hepatic Th2 immune response as a modulator of key molecules in the RAS pathway, such as ACE. This knowledge suggests that cytokines IL-4, IL-10, and/or IL-13 could be potential biomarkers for predicting the onset of SAH in individuals with MASLD and could also lead to new therapeutic strategies to reduce the incidence of SAH in patients with MASLD comorbidities that are alarmingly increasing globally.

109. 092. **UNVEILING METABOLIC MECHANISMS UNDERLYING TRIIODOTHYRONINE-MEDIATED DENDRITIC CELL ACTIVATION**

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The thyroid hormone (TH) triiodothyronine (T3) induces dendritic cells (DC) activation and maturation. We demonstrated that T3-treated DC (T3-DC) promote pro-inflammatory T cell responses and enhance cytotoxic T cells activity both in vitro and in vivo, improving anti-tumor responses in T3-DC vaccine murine models. Considering the main role of TH metabolism regulation, we aimed to assess T3 effects on DC metabolic program and its impact on maturation. DC were differentiated in vitro from C57BL/6 mice bone marrow progenitors with GM-CSF for 8 days (GM-DC). GM-DC were treated with T3 (10nM, 18h), L-NAME (5mM, 18h) or left untreated (control). Glycolytic

metabolism was assessed by measuring glucose and lactate levels in supernatants (SN) and cellular respiration by oxygen consumption (OCR) using high-resolution respirometry. Protein expression of p-mTOR (Ser2448), mTOR, HIF1alfa and iNOS was determined by Western Blot. Nitrite levels (nitric oxide –NO- production) were measured by Griess reaction in SN. DC activation and maturation markers (CD86, MHC-II, IL-12p40p70) were determined by FACS. Statistical analysis was performed using T-test or two-way ANOVA with Tukey's multiple comparison test ($p<0.05$). T3 increased glucose consumption and lactate production in GM-DC ($p<0.01$) while decreasing mitochondrial respiration ($p<0.01$). However, extra-mitochondria OCR was elevated in T3-GM-DC ($p<0.001$). T3 also induced iNOS expression and NO production ($p<0.0001$). Inhibition of iNOS with L-NAME restored mitochondrial respiration ($p<0.05$) while maintaining enhanced glycolysis ($p<0.01$) and high expression of activation and maturation markers. Regarding the mechanism involved in these metabolic changes, T3 induced HIF1alfa expression ($p<0.001$), which was dependent of iNOS activity, as HIF1alfa was not detected in L-NAME-treated T3-GM-DC. No changes were registered in p-mTOR/mTOR expression after T3 treatment. In conclusion, T3 promotes glycolysis while reducing mitochondrial bioenergetics in GM-DCs via a HIF1alfa/iNOS-dependent mechanism. These results and further research could provide new strategies to modulate the immunogenic potential of DC with therapeutic purposes.

110. 148. **ROLE OF GLUTAMINOLYSIS IN ACTIVATED CD4+ T LYMPHOCYTES IN THE PRESENCE OF VITAMIN D**

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Vitamin D (VitD) is an essential nutrient that plays a crucial role in the immune system. It modulate several immune cells, including $CD4^+$ T helper lymphocytes. The expression of the VitD receptor appears after T cell activation, coinciding with a shift in T cell metabolism primarily towards glycolisis. Interestingly, previous studies indicate that later after activation, VitD promotes the proliferation and survival of $CD4^+$ T lymphocytes, however it also reduces glycolysis, suggesting that an alternative metabolic pathway must be compensating the energetic requirements. Glutaminolysis is a mitochondrial pathway responsible for generating cellular energy and supporting cell proliferation, however it is unknown whether Glutaminolysis is modulate by VitD. Our aim was to determine the effect of VitD on the glutaminolysis pathway in $CD4^+$ T cells. $CD4^+$ T cells were isolated from peripheral blood and cultured in RPMI1640 medium for 4 days at 37°C in the absence or presence of VitD (10nM). Viability and cell count were evaluated in the presence of transport and enzymatic pathway inhibitors of glutamine. Along with glucose uptake using 2-NBDG and lactate production, proteomic analysis, transporter expression, and characterization of kinetic parameters of glutamine transport were performed. A significant decrease in glucose uptake and lactate production, and an increase in viability and cell count were observed. Furthermore, proteomic analysis revealed elevated expression of glutaminolysis pathway enzymes; glutaminase (GLS) and glutamate dehydrogenase (GDH) in the presence of VitD ($p<0.05$). Additionally, the inhibition of the glutaminolysis pathway in the presence of VitD decreased cell count when compared to control samples. Finally, the kinetics of H^3 glutamine transport in the presence of VitD showed a significant increase in the activity constant of glutamine transport in the presence of VitD. Our data showed that glutamine plays a relevant metabolic role for $CD4^+$ T lymphocytes activated in the presence of VitD.

111. 204. **LIPID METABOLISM MODULATES DENDRITIC CELL ACTIVATION, INFLAMMATORY RESPONSE AND BACTERIAL BURDEN DURING M. BOVIS BCG INFECTION**

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Tuberculosis represents a major challenge to public health, killing more than 1 million people

each year. In the lungs, *Mycobacterium tuberculosis* (Mtb) infects mainly macrophages and dendritic cells (DCs), which are important bacterial reservoirs and initiators of the immune response. Mtb changes host metabolism as a survival mechanism. Lipid accumulation has been shown to be a key component in host–pathogen interactions, enabling bacterial persistence. Thus, herein, we evaluated the role of lipid synthesis enzymes in DC activation during *M. bovis* BCG infection. DCs obtained from C57BL/6 bone marrow were differentiated with 10 ng/ml GM-CSF for 10 days and infected with BCG (MOI 5) or Mtb H37Rv (MOI 1). Unlike macrophages, Mtb- and BCG-infected DCs presented a late increase in lipid droplets (LD) and in lipid-related (*fasn, dgat1, dgat2, acat1, plin2, plin3, atgl*) and proinflammatory (*il1b, il10, cox2, 5lo, mr1*) gene expression. Indeed, triacylglycerol (TAG) and cholesterol ester (CE) accumulate in DCs during Mtb and BCG infection. To evaluate the roles of TAG and CE synthesis, BCG-infected DCs were treated with diacylglycerol acyltransferase 1 (DGAT-1; A922550; 20 μM) and acyl-coenzyme A (CoA):cholesterol acyltransferase (ACAT; Ci976; 10 μM) inhibitors. ACAT inhibition did not affect LD formation in DCs, but it increased the bacterial burden. Furthermore, ACAT inhibition reduced the production of IL-1beta, IL-6, IL- 10, IL-12p40, TNF-alpha and PGE_2 by BCG-infected DCs. DGAT-1 inhibition only reduced LD accumulation and PGE_2 release by these cells. We also observed that the treatments inhibited the expression of MHCI, MHCII and CD80 by infected DCs. TAG and DGAT-1 are important for LD formation in DCs, whereas CE synthesis by ACAT is involved in the host inflammatory response. Both pathways seem to represent potential targets to modulate the DC response against tuberculosis.

112. 288. METABOLOMIC CHANGES INDUCED BY A GASTROINTESTINAL HELMINTH INFECTION IN MULTIPLE SCLEROSIS PATIENTS

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Background: Multiple sclerosis (MS) is an autoimmune demyelinating disease with a growing global prevalence in industrialized countries. Evidence suggests that MS patients that naturally acquired helminth infections (MS+HI) ameliorate disease symptoms and reduced disability scores, possibly through immunomodulatory mechanisms. Additionally, immunometabolism field underscores the bidirectional relationship between immune function and metabolic processes. We hypothesized that the MS+HI condition presents differential metabolomic profile than MS patients promoting immunoregulatory pathways. **Objectives:** We aim to characterize the plasma metabolomic profile of MS and MS+HI patients to identify potential immunoregulatory metabolites. **Methods:** Untargeted plasma metabolomics was conducted on 30 MS and 17 MS+HI patients using mass spectrometry-based metabolic screening (CE-FTMS). Differentially expressed metabolites (DEMs) between the groups were identified through univariate statistical analyses. A t-test was mapped robustly within each metabolite and between groups, and the cut-off value was set at 0.05. Over-representation analysis (ORA) with up-regulated metabolites was implemented using the hypergeometric test to evaluate whether a particular metabolite set is represented more than expected by chance. **Results:** Out of the 483 identified metabolites, 52 showed differential expression between the groups. 45 were more abundant in the MS+HI group, including kynurenines, polyamines, amino acids, ɣ-glutamyl peptides, among others. The ORA revealed over-representation of several pathways such as nicotinate and nicotinamide metabolism, cysteine and methionine catabolism, tryptophan metabolism and methylation pathways in MS+HI patients. **Conclusion:** Our results suggest that helminth infection alter metabolic pathways and may contribute to the trained immune protective response and clinical benefits in MS patients. Further research is needed to validate these metabolites as potential immunoregulatory molecules.

113. 293. PLASMA FROM PULMONARY TUBERCULOSIS PATIENTS IMPACTS ON MONOCYTE METABOLISM DETERMINING THEIR ABILITY TO GENERATE MIGRATORY DENDRITIC CELLS

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Mycobacterium tuberculosis (Mtb) interferes with dendritic cell (DC) functions, impairing the onset and development of adaptive immunity, which ultimately favors the progression of tuberculosis (TB). While our previous research has shown that the activation of the glycolysis-HIF1A axis is necessary for DCs to acquire a migratory phenotype, we found that early activation of this pathway in monocytes can have detrimental effects on the migratory capacity of subsequently differentiated DCs. Therefore, the aim of this study is to elucidate the mechanisms underlying the exacerbated glycolysis observed in monocytes during TB. We hypothesize that plasma from TB patients activates the HIF1A/glycolysis axis in monocytes. To test this hypothesis, we generated pools of decomplemented plasma from either TB patients (pTB) or healthy subjects (pHS) and added them to monocytes isolated from healthy donors for one hour. Under these conditions, we observed no evidence of cell death. Additionally, no differences were observed in the initial levels of lactate, glucose, and triglycerides in pTB and pHS. Using SCENITH technology, we found that the CD16+ monocyte subsets exposed to pTB exhibited an increased glycolytic capacity compared to those incubated with pHS ($p<0.05$). Consistent with these findings, TB plasma also induced a significant increase in lactate production ($p<0.05$) alongside elevated expression of HIF1A ($p<0.05$). Notably, DCs derived from monocytes exposed to pTB exhibited a reduced ability to migrate through a collagen matrix ($p<0.05$). We therefore decided to assess whether activation via the IgG Fc receptor CD16 (FcγRIIIa) might be involved and observed that if IgG was depleted, the pTB-driven induction of lactate and HIF1A disappeared ($p<0.05$). In conclusion, we have shown that TB plasma influences CD16+ monocyte metabolism, involving IgG immune complexes, suggesting that modulation of this process could serve as a promising strategy for developing therapeutic approaches to manipulate immune responses.

114. 299. PREGNANCY PROMOTES MATERNAL MONOCYTE FATTY ACID METABOLISM. ROLE OF LIPID UTILIZATION IN TROPHOBLAST-MEDIATED M2-LIKE PROFILE MACROPHAGES

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Cellular metabolism influences immune cell state and fate, contributing to organismal homeostasis. In normal pregnancy, extravillous trophoblast cells orchestrate the recruitment of monocytes and their differentiation into M2-like decidual macrophages. Failures in these processes lead to pregnancy complications. Immunometabolic role during early pregnancy has remained unexplored until recently. By studying monocytes from pregnant women at 16-20 weeks (16-20w), we reported increased glucose dependence and enhanced efferocytosis inhibited by the glycolysis inhibitor 2-deoxy-D-glucose (2DG). Interestingly, this inhibition was more pronounced when 2-DG was combined with the oxidative phosphorylation inhibitor Rotenone. Here, our aim was to characterize monocyte lipid metabolism during normal pregnancy, and its contribution to the acquisition of an M2-like profile. Peripheral blood mononuclear cells isolated from fertile non-pregnant and 16-20w pregnant women by Ficoll-Paque were analysed for long chain fatty acid (LCFA) up-

take and lipid droplets (LD) by Bodipy-FL-C12 or Bodipy493/503 and flow cytometry. For *in vitro* designs, monocyte-derived macrophages (M0) from non-pregnant women were incubated with trophoblast conditioned media (Tb-CM) from HTR8/SVneo cell line +/- metabolic inhibitors 2DG, oligomycin and etomoxir. ATP production, gene expression, lipid droplet localization and cell profile were analyzed by bioluminescent assay, RT-qPCR, confocal microscopy and flow cytometry, respectively. Monocytes from pregnant women present not only increased glucose dependence but also increased LCFAs uptake ($p<0.05$) without changes in LD accumulation. In the *in vitro* model, the incubation of M0 with Tb-CM increased CPT1 FAO-rate limiting step importer ($p<0.05$) and the colocalization of lipid droplets with mitochondria (Manders M1/M2, $p<0.05$). ATP production in this setting was impaired by the three inhibitors separately and their combination produced greater effect ($p<0.05$). Moreover, etomoxir prevented CD209 increase ($p<0.05$) and tended to boost CD86 expression in M0 upon Tb-CM treatment. Our results indicate that pregnancy increases maternal monocyte lipid utilization associated to an M2-like profile of decidual macrophages.

115. 315. **THE ROLE OF VISFATIN IN INFLAMMATORY PATHWAYS OF NLRP3 INFLAMMASOME ACTIVATION IN CELLULAR MODELS**

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Background: Abdominal obesity, a key characteristic of T2DM, is increasingly prevalent in younger adults. As these individuals age, the chronic low-grade inflammation associated with visceral obesity contributes to the concept of inflammaging, affecting organ structure and function. Key pathways involved include cellular senescence, NLRP3 inflammasome activation, and oxidative stress. Obesity-induced chronic inflammation leads to the release of various adipokines, which contribute to vascular endothelial dysfunction, hypertension, and atherogenic dyslipidemia. Visfatin is one such adipokine, but its role remains relatively unexplored. **Objectives:** This study aims to evaluate the actions of Visfatin to describe the role in NLRP3 inflammasome activation pathways in cellular models. **Methods:** RAW 264.7 (cultured in DMEM supplemented with 10% FBS) and HUVEC (cultured in Endothelial Cell Growth Media) cells were cultured and divided into treatment groups (LPS, Visfatin, LPS+Visfatin) and a control. Experiments were conducted at 6, 12, and 24 hours. mRNA levels of TNF-α, IL-6, iNOS, IL-1β, and NLRP3 were evaluated by RT-PCR. Nitrite levels were assessed using the Griess method, and cell migration was analyzed via wound healing assay. **Results:** At 6 hours, IL-1β and IL-6 expression increased with LPS and LPS+Visfatin. At 12 hours, IL-1β and iNOS levels rose with Visfatin treatment, while at 24 hours, TNF-α and IL-6 were elevated in the LPS group. Visfatin+LPS-treated cells exhibited higher proliferation and migration, akin to control. Nitrite levels increased in LPS-treated cells at 6 hours and in Visfatin-treated cells at 12 hours. **Conclusion:** Preliminary results suggest that Visfatin induces inflammatory responses in monocytes, leading to polarization towards an inflammatory phenotype, characterized by an increased inflammatory markers (IL-1β, iNOS) and nitrite production.

116. 341. **MONOCYTES REACHING THE PLEURAL CAVITY BECOME LESS GLYCOLYTIC AND SECRETE PRO-RESOLVING LIPID MEDIATORS**

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a major global health chal-

lenge. Despite advancements in TB control, our understanding of the immune response to this pathogen is still incomplete. Our previous research indicated that pro-inflammatory (M1) macrophages exposed to acellular pleural effusions from TB patients (TB-PE) display reduced glycolytic activity due to the downregulation of hypoxia-inducible factor 1A (HIF1A), which impairs bacterial resistance. This reduction is driven by polyunsaturated fatty acid metabolites present in TB-PE. In this study, we aimed to identify these lipids and their potential sources in the TB-affected pleura. Lipidomic analysis of pleural effusions from TB patients revealed high levels of omega- 3-derived pro-resolving lipid mediators (SPMs), such as 18-HEPE, 7(R)-Maresin 1, ProtectinDx, and Resolvin D5, which were associated with inhibited glycolysis in M1 macrophages ($p<0.05$). Single-cell RNA sequencing indicated upregulation of ALOX5 in pleural monocytes compared to circulating monocytes from TB patients ($p=6x10$□□), suggesting enhanced biosynthesis of lipoxins and resolvins in the tuberculous pleural environment. Furthermore, significant amounts of RvD5, 18-HEPE, and PDx were detected in extracts from monocytes treated in vitro with TB-PE ($p<0.05$), reinforcing the idea that CD14+ cells produce SPMs in response to the pleural TB environment. Consistent with this, we observed reduced ex vivo glycolytic capacity in CD14+ cells residing in the TB pleural cavity compared to circulating monocytes, as measured by SCENITH analysis ($p<0.05$), suggesting that once phagocytes enter the pleural cavity, their glycolytic activity is diminished by the local microenvironment. Notably, a significant increase in glycolysis was observed in pleural macrophages from TB patients undergoing antibiotic treatment compared to untreated patients ($p<0.05$). These findings support the notion that active mechanisms obstructing the long-term protective immune response within the pleural cavity are likely mediated by SPMs, providing an alternative explanation for the frequent relapses observed in untreated pleural TB patients.

117. 364. **THE CD43 SIALOMUCIN PROMOTES GLUT-1 EXPRESSION IN T CELLS**

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CD43 is a type I transmembrane sialomucin abundantly expressed in T lymphocytes. In normal T cells, CD43 delivers co-stimulatory signals implicated in thymocyte selection, maturation, migration, adhesion, and activation of mature T cells, and its expression in tumor cells is associated with a poor prognosis. We hypothesized that the diverse functions of CD43 are attributed to its long and rigid structure, which positions it as one of the initial molecules conveying environmental cues necessary for cell survival and proliferation. Specifically, we investigated whether CD43- mediated signals participate in the metabolic adaptations that T cells undergo in response to activation conditions where glycolysis supplies the bioenergetic demands of proliferation. We evaluated the expression levels of the glucose transporter GLUT-1 as well as the glycolysis and glutaminolysis rates in normal human peripheral blood CD4+ T cells and Jurkat cells (Acute lymphocytic leukemia T lymphoblast) following stimulation with TCR+CD43, TCR+CD28, CD43, or CD28. At 48h post-stimulation, GLUT-1 membrane expression in CD4+ T cells increased ~10-fold in response to the TCR+CD43 stimulus compared to unstimulated cells, surpassing the TCR+CD28 stimulus. On the contrary, Jurkat cells exhibited increased total and membrane GLUT-1 expression only when stimulated with CD43 or CD28 alone but not with the TCR+CD43, TCR+CD28 stimuli. Interestingly, no increase in glucose intake was detected, while lactate levels decreased, and glutamine intake, but not glutamate release, increased. Moreover, investigating whether the expression of CD43 correlated with Glut-1 expression in different types of cancer with the GEPIA2 platform revealed a positive correlation between CD43 and Glut-1 in acute myeloid leukemia. Altogether, our data uncover a role for CD43- dependent signaling in promoting Glut-1 expression in both normal T cells and lymphoid tumor cells, expanding the functions of this molecule.

118. 401. **ADIPONECTIN AND ITS PREDICTIVE VALUE IN METABOLIC SYNDROME: INSIGHTS FROM PANAMA**

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Recent data indicate that metabolic syndrome (MetS) is becoming increasingly prevalent in Panama, contributing to the rise of various health problems, including cardiovascular diseases and diabetes. Adiponectin, a cytokine that circulates in plasma. Its concentration varies depending on an individual's body mass index and sex. Unlike other adipokines, the plasma concentration of adiponectin is inversely related to body fat content and obesity. One of the key functions of adiponectin is its ability to improve insulin sensitivity in skeletal muscles, which is particularly relevant in the development of MetS. Additionally, adiponectin has anti-inflammatory properties that can counteract the low-grade chronic inflammation commonly associated with obesity and MetS. An increase in visceral fat mass leads to a reduction in systemic adiponectin levels, contributing to insulin resistance and other components of MetS. This decrease in adiponectin levels is closely linked to the worsening of metabolic syndrome symptoms, suggesting a potential predictive value for this biomarker. **The objective** of this study is to correlate serum adiponectin levels in a Panamanian population with metabolic syndrome. **Methodology:** Serum ADIPOQ levels were evaluated using chemiluminescence methods in subjects diagnosed with metabolic syndrome and a control group. **Results:** The results showed a significant downward trend in ADIPOQ levels among subjects with MetS, reinforcing the hypothesis of a crucial connection between adiponectin levels and this condition. Contributing to a deeper understanding of adiponectin's role in MetS, inflammation, and other related health conditions. **Conclusions:** adiponectin, could be a useful biomarker for assessing the risk of MetS and that interventions aimed at increasing its levels could be effective in reducing this risk. The negative correlation between adiponectin and triglycerides suggests a protective role of this protein against hypertriglyceridemia, while the positive correlation with HDL cholesterol indicates that higher adiponectin levels are associated with a healthier lipid profile.

119. 436. MODULATION OF MACROPHAGE RESPONSES TO MYCOBACTERIAL INFECTION BY CB2 RECEPTOR AGONIST: EFFECTS ON INFLAMMATORY MEDIATORS AND LIPID METABOLISM

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Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis* (Mtb), primarily affecting humans. Mtb represents a major cause of infection-related mortality globally. The interactions between macrophages and mycobacteria are critical for either controlling or establishing infection. Macrophage activation triggers inflammatory cell recruitment, potentially leading to granuloma formation or bacterial eradication. Recent strategies have proposed host-directed therapies, focusing on modulating the host response rather than directly targeting the bacteria. The endocannabinoid system (ECS) has been identified as a potential target for modulating various pathophysiological conditions, including respiratory diseases. Given the challenges posed by immune evasion mechanisms and antibiotic resistance, there is a pressing need to enhance our understanding of TB and explore alternative treatment modalities. In this context, our study aims to evaluate the effects of the CB2-selective agonist GP1a on *M. bovis* BCG-induced macrophage activation. To this end, J774A.1 macrophages were pretreated with GP1a for 30 minutes, followed by stimulation with irradiated *M. bovis* BCG (iBCG) at a multiplicity of infection (MOI) of 3 for 1 hour. We observed an increased expression of CB2 in macrophages upon iBCG-stimulation within 1 and 6 hours. Additionally, GP1a treatment (10 μM) inhibited the iBCG-induced production of inflammatory mediators, including TNF-α, prostaglandin (PG)E_2, IL-10, IL-6, nitrite, and ciclooxigenase (COX)-2. GP1a treatment also decreased the transcription of pro-inflammatory genes (*inos, il1b, cox2*) and genes related to lipid metabolism (*dgat1, acat1, plin2, atgl, cd36*), as measured by quantitative PCR (qPCR). Lipid droplet (LD) accumulation in macrophages, induced by BCG infection, supports bacterial survival. We observed that iBCG-stimulated macrophages exhibited an increase in the number of LDs per cell, which was reduced by GP1a pretreatment. Moreover, CB2 antagonist AM630 (200 nM) reverted GP1a effects on lipid accumulation in stimulated macrophages. Furthermore, we assessed the effects of GP1a pretreatment on toll-like receptor (TLR) expression and signaling pathways. GP1a pre-

treatment did not alter the expression of TLR2 or TLR4. However, CB2 agonist pretreatment significantly inhibited NF-κB translocation to the nucleus and reduced the activation of inflammatory signaling pathways, including NF-κB, ERK 1/2, and p38 MAPK, but did not affect JNK signaling. In conclusion, activation of CB2 by GP1a modulates the macrophage response to iBCG by reducing inflammatory mediator levels. GP1a also influences lipid metabolism and downregulates inflammatory signaling pathways. These findings underscore the potential of CB2 agonists as therapeutic targets for tuberculosis and highlight the need for further research. Our results are consistent with existing literature suggesting that GP1a is a selective immunosuppressive CB2 agonist. In summary, our study demonstrates that the CB2 agonist GP1a modulates the macrophage response to mycobacterial stimulation by attenuating the NF-κB signaling pathway, inhibiting inflammatory mediators, and altering metabolic processes and lipid accumulation. Collectively, our data suggest that CB2 may be a promising candidate for novel host-directed immunotherapy against mycobacterial infections, warranting further investigation.

120. 449. **PREGNANCY ENTAILS A METABOLIC REWIRING OF MATERNAL CIRCULATING NEUTROPHILS**

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Trophoblast cells interact with maternal immune cells at placentation favoring an anti-inflammatory microenvironment required for fetal growth. Circulating neutrophils appear activated throughout normal pregnancy and even more in pregnancies complicated by preeclampsia. While metabolic reprogramming is known to shape the functional profile of immune cells in a number of settings, the immunometabolic rewiring of neutrophils during pregnancy has not yet been explored. **The aim** of this work was to deepen into the metabolic reprogramming of neutrophils throughout pregnancy. Peripheral blood neutrophils from healthy non-pregnant women (NP-PMN), 16-20 weeks' pregnant (16-20w-PMN) and from women at term (Term-PMN) were analyzed *ex vivo* to compare their metabolic profile. The effect of trophoblast factors on NP-PMN metabolic state was investigated *in vitro* using first trimester trophoblast-derived cell line (HTR-8/SVneo) conditioned media. RT-qPCR and fluorescent probes (2-NBDG, BODIPY-493/503, BODIPY-FL-C12, MitoSpy/MitoTracker) were used to assess the PMN metabolic profile by flow cytometry. For functional assays, PMN migration, reactive oxygen species (ROS) production and neutrophil extracellular trap (NET) formation were evaluated. Trophoblast cells-derived factors induced an increase in glucose uptake and lipid droplet accumulation in NP-PMN ($P<0.05$). Accordingly, 16-20w-PMN showed an increase in glucose uptake and lipid droplet formation, compared to NP-PMN, accompanied by a higher release of PMA-induced NETs. Interestingly, this effect was blocked by etomoxir, a fatty acid oxidation inhibitor (all $P<0.05$). Both 16-20w-PMN and Term-PMN, presented higher basal levels of ROS production compared to NP-PMN. However, PMA-triggered ROS production was restricted in PMN from both pregnancy groups respect to NP-PMN ($P<0.05$). Term-PMN presented the highest increase in PMA-induced glucose uptake of the three groups ($P<0.05$).
Our results support that the functional shaping of neutrophils along pregnancy is accompanied by immunometabolic reprogramming and that first trimester trophoblast cells contribute to this regulation at early pregnancy.

121. 454. **METABOLIC REPROGRAMMING MECHANISMS MEDIATED BY SREBPS DURING ZIKA VIRUS INFECTION**

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Zika virus (ZIKV) infection is a global public health issue, linked to neurological disorders in adults and congenital diseases in newborns. ZIKV shows a strong tropism for neuronal cells, disrupting development and causing cell death. Several RNA viruses manipulate lipid metabolism and exploit lipid droplets (LDs) to enhance viral replication and pathogenesis. LDs, crucial for lipid metabolism, energy regulation, intracellular transport, are also involved in infections and inflammation. However, the mechanisms underlying LD formation and their role in ZIKV infection in neural cells remain unclear. This study aims to investigate the role of the transcription factor SREBP in lipid metabolism reprogramming during ZIKV infection in neural cells. Using human SH-SY5Y neural progenitor cells, we investigated the impact of pharmacological (Betulin) and genetic inhibition of SREBPs on lipid metabolism and cellular dysfunctions induced by ZIKV infection. Our results show that ZIKV induces lipid metabolism remodeling by upregulating key lipid metabolism proteins like PLIN-2, DGAT-1 and FASN, and increasing SREBP-1 activation, contributing to the accumulation of LDs observed in SH- SY5Y cells after infection. Inhibition of SREBP processing by Betulin reduced LD biogenesis, decreased the expression of the assessed proteins, and negatively impacted ZIKV replication in neural cells. Additionally, silencing SREBP-1 and SREBP-2 genes in SH-SY5Y cells also suggested a reduction in ZIKV replication. ZIKV-infected neural cells showed increased ROS production and mitochondrial dysfunction, which likely contribute to the observed cellular alterations. Pharmacological inhibition of SREBPs maturation reduced mitochondrial superoxide production, descreased caspase-1 activation, and protected neural cells from ZIKV-induced cell death. These findings highlight the involvement of SREBPs in lipid metabolic reprogramming that contributes ZIKV replication and pathogenesis, opening new perspectives for antiviral therapeutics development.

122. 455. REGULATION OF LIPID METABOLISM IN THE ACTIVATION OF THE INFLAMMASOME DURING SARS-COV-2 INFECTION

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Background and objective: Viruses exploit host lipid metabolism to promote their replicative cycle, and SREBPs play a regulatory role in fatty acid and cholesterol metabolism. Additionally, SREBPs have been linked to lipid droplet (LD) biogenesis. Our group discovery that SARS-CoV-2 infection induces LD biogenesis in human monocytes. Furthermore, SREBPs are also involved in caspase-1 activation and cell death through pyroptosis. However, the role of SREBPs during SARS-CoV-2 infection remains largely unexplored. Thus, our hypothesis is that SREBPs regulate host metabolism, influencing SARS-CoV-2 replication. In this study, we investigated the impact of pharmacological and molecular inhibition of SREBPs on lipid metabolism during SARS-CoV-2 infection. **Methods and results:** Human epithelial pneumocytes (calu-3) cells were infected with 0.01 MOI of SARS-CoV-2 for 24 or 48 hours. We found that infection leads to the expression and activation of SREBP1 and SREBP2, resulting in the upregulation of genes involved in lipid metabolism and cytokines. This, in turn, leads to the accumulation of triglycerides, cholesterol, and LD biogenesis. Partial inhibition of SARS-CoV-2 replication and cell death was observed when either SREBP1 or SREBP2 was knocked down. Interestingly, combined knockdown of SREBP1 and SREBP2 demonstrated synergistic inhibition, downregulating lipid and cytokine genes. This led to a reduction of viral replication and the LD formation. Furthermore, we used the pharmacological inhibitor fatostatin, which effectively inhibited the activation of SREBPs, LD formation by reducing the proteins DGAT1 and PLIN2, and viral replication. Notably, SREBPs and DGAT1 inhibition blocks the inflammasome activation by reduce the activation of caspase-1, gasdermin D1, and the release of IL-1β and IL-18. **Conclusion:** Collectively, our data highlight the crucial role of SREBPs as master regulators during SARS-CoV-2 infection. Inhibiting these factors could prove to be critical in combating viral infection.

123. 483. TARGETING ACYL-CoA:CHOLESTEROL ACYLTRANSFERASE DURING SARS-CoV-2 INFECTION

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Lipid droplets (LDs) play essential roles in the storage and metabolism of neutral lipids, such as triacylglycerol and cholesterol esters, as well as in cell signaling, infection, and inflammation processes. Intracellular cholesterol is esterified by acyl-CoA acyltransferase (ACAT) or Sterol O-acyltransferase (SOAT) and subsequently stored in LDs. Cholesterol homeostasis is critical for various stages of the viral life cycle, including entry, replication, assembly, and release. Metabolic syndrome and hyperlipidemia have been linked to worse outcomes in SARS-CoV-2 infection, and cholesterol-lowering drugs like statins may enhance COVID-19 survival rates, underscoring the potential of targeting cholesterol metabolism as a therapeutic approach. In this study, we investigated the significance of the final step of cholesterol ester synthesis by inhibiting the ACAT enzyme both genetically and pharmacologically in a SARS-CoV-2-infected lung epithelial cell line (Calu-3). Following infection, there was an increase in lipid droplets and alterations in cholesterol metabolism. We examined the expression of SOAT-1 and SOAT-2, finding that SOAT-2 was more upregulated than the SOAT-1 isoform. Through siRNA-mediated knockdown of both isoforms, we demonstrated that SOAT-2 had a greater impact on SARS-CoV-2 replication. Additionally, using an ACAT inhibitor (CI- 976) as an antiviral treatment, we successfully suppressed viral replication, cytokine release, antiviral response, and PANoptosis-induced cell death, reversing the activation of caspase 1 and MLKL. Our findings reveal that SARS-CoV-2 replication relies heavily on the activation of lipid metabolism, accumulation of LDs, and cholesterol, particularly via the SOAT-2 enzyme. Viral infection triggered a pronounced inflammatory and antiviral response along with PANoptosis cell death. Treatment with the ACAT inhibitor effectively prevented LD biogenesis, cholesterol accumulation, viral replication, chemokine release, and PANoptosis-induced cell death. These results highlight the crucial role of ACAT, cholesterol metabolism, and LDs in viral replication and cell death, suggesting that their modulation could serve as a promising antiviral strategy.

124. 488. SEX DIFFERENCES IN THE EFFECT OF EXTRACELLULAR VESICLES FROM HUMAN TERM PLACENTAL VILLI ON MACROPHAGE IMMUNOMETABOLISM AND ENDOTHELIAL CELL FUNCTION

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Aims: During pregnancy the placenta releases soluble factors that contribute to its function and fetal development, including extracellular vesicles (EVs). However, the specific influence of EVs according to fetal sex remains unclear. We have previously demonstrated a sex-differences in the metabolic profile of placental macrophages (HBC) at term. Our aim is to investigate the sex-specific contribution of placental conditioned media (CM) or isolated EVs to differential macrophage (MA) metabolism and endothelial cell (EC) migration **Methodology:** EVs were isolated from placenta villi explant (PVE) CM by differential centrifugation and characterized. Monocytes from healthy female donors' peripheral blood were differentiated into MA with GM-CSF and cultured with CM or EVs from male (M) or female (F) PVE to assess their phenotypic and metabolic profile by flow cytometry. HBC were obtained from normal term placenta by enzymatic digestion. HUVEC were used to study endothelial cell migration. Sex-differences in macrophage metabolic profile were compared through bioinformatic assays using public database (accession GSE30595) by gseapy package in Python. **Results:** CM from F-PVE polarized MA to a stronger

antiinflammatory phenotype increasing almost 2 times the production of CD163, CD206, CD209 and IL-10 secretion respect to M-PVE (P<0.05). Only F-CM increased lipid droplets accumulation (P<0.05) without changing glucose uptake. Similar results were observed with the isolated EVs from F-placenta. Consistently, HBC obtained from F-placenta were more prompted to an antiinflammatory phenotype and presented increased lipid metabolism compared to M. Furthermore, only F-EVs increased endothelial cell migration (P<0.05). MA from M volunteers were more activated to a classical phenotype than F by bioinformatic studies as well. **Conclusions:** Our results suggest that placental sex-specific EVs play a differential regulatory role in macrophage phenotype and metabolism, aligning with the immunometabolic profile of HBC. Additionally, these findings imply a potential involvement of sex-specific EVs in endothelial cell function.

125. 522. **EXPLORING MITOCHONDRIAL DYNAMICS IN TRYPANOSOMA CRUZI-INFECTED MACROPHAGES**

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Macrophages are the first defense against *Trypanosoma cruzi*, the agent of Chagas Disease. Despite this, *T. cruzi* exploits macrophages for survival and replication. Mitochondria play a crucial role in metabolism and immune function through dynamic fission and fusion processes. In macrophages, fission links to the M1 while fusion correlates with M2 profile. We hypothesize that *T. cruzi* infection alters mitochondrial metabolism in macrophages, affecting their response to support parasite proliferation. However, the role of mitochondrial dynamics during *T. cruzi* infection is poorly understood. Bone marrow-derived macrophages, peritoneal macrophages, and RAW cells were infected overnight with trypomastigotes (Tp) from the Tulahuen strain (1:5 cell/Tp ratio). Macrophages were pre-treated with Mitochondrial Division Inhibitor-1 (Mdivi-1) or carbonyl cyanide m-chlorophenylhydrazone (CCCP) to inhibit or induce mitochondrial fission, respectively, before infection. Mitochondrial morphology was assessed by confocal microscopy using MitoSpyOrange, with image analysis by ImageJ's MiNA plugin. Western blotting evaluated mitochondrial fusion proteins MFN1 and OPA1, as well as Arginase-1 and iNOS expression. Parasite numbers were quantified by immunofluorescence with InCarta software. Cell viability was measured by LDH activity. Statistical analysis involved t-tests and one-way ANOVA. Infection increased the number and extent of mitochondrial branches, peaking at 30 hours post-infection (p<0.05; p<0.0005), indicating mitochondrial fusion. This was correlated with higher levels of MFN1 and OPA1, coinciding with increased iNOS and Arginase-1 expression, while reduced OPA1 and MFN1 expression was observed in CCCP-treated macrophages. Treatment with Mdivi-1 led to fused mitochondrial morphology compared to DMSO-treated controls (p<0.0001). However, no significant differences were observed in the infection rate or the number of parasites between infected macrophages previously treated with CCCP or Mdivi-1 and the control group, while cell viability was not affected in any condition. *T. cruzi* infection modifies mitochondrial morphology, but further research is needed to fully understand its impact on macrophage polarization and infection dynamics.

126. 523. **IMPACT OF HIGH-INTENSITY INTERVAL TRAINING ON TREG CELLS AND INSULIN SENSITIVITY IN AGING MICE**

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Aging is a natural process that leads to physiological changes like insulin resistance, increased adipose tissue, and alterations in Treg cells, which are essential for energy balance and insulin sensitivity in visceral fat. Physical exercise, especially high-intensity interval training (HIIT), is an effective non-pharmacological intervention in treating and preventing chronic diseases due to its anti-inflammatory effects. This study aims to evaluate the impact of HIIT on Treg cells in elderly mice using C57BL/6-Tg(Foxp3-GFP) animals. It is a controlled study with three groups: trained elderly mice (12 months old), untrained elderly mice, and untrained young mice (2 months). Train-

ing occurred three times per week for 16 weeks, with 15-min sessions. Each session started with a 3-min warm-up (50% of maximum speed), followed by 1 min of HIIT (85%-100% of maximum speed) and 1 min of recovery. Untrained groups performed one exercise session (10m/s) for 10 min weekly. Intraperitoneal glucose and insulin tests were performed both before and after the intervention. Treg cells' number and cytokine profile in adipose tissue, skeletal muscle, and popliteal lymph nodes were analyzed in trained and non-trained animals. Results showed that the percentage of Treg cells in the popliteal lymph node of untrained elderly controls was 42% higher than in young controls. While in the trained group, this difference dropped to 7%. Furthermore, our results showed differences in the glycemi curve of the insulin sensitivity test, about 21% compared to pre-intervention, while no difference was observed in other groups. The study will contribute to a better understanding of the mechanisms involved in improving insulin sensitivity through exercise during aging.

127. 602. **IGA LEPTIN-REACTIVE ANTIBODIES DIFFER BETWEEN INDIVIDUALS FOLLOWING AN OMNIVOROUS OR A PLANT-BASED DIET**

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Background. Low-affinity leptin reactive-antibodies are present in healthy subjects and altered in individuals with eating disorders, but its regulation by diet has not been explored. **Objectives:** This study aimed to analyze the circulating levels of anti-leptin IgA and IgG antibodies in adults who follow a plant based or an omnivorous diet. **Methods:** This cross-sectional study was conducted in 89 adults (18-50 years) categorized in two groups: omnivorous diet (n=56) and plant base diet (including vegetarians, lacto-ovo vegetarians and vegans, n=33). A food consumption frequency questionnaire validated for Mexican population was applied to dietary assessment. Body composition parameters (BMI, body fat) and biochemical variables (glucose, triglycerides, total cholesterol and leptin) were evaluated. An in-house ELISA test was performed to quantify IgA 's and IgG´s leptin reactive antibodies in their different fractions (free, total and immune complexes percentage). The ratio of free, total and immune complexes leptin antibodies of IgA/IgG were also evaluated. **Results:** IgA leptin reactive antibodies levels, both on its total fraction and the immune complexes fraction were higher in omnivorous versus individuals following a plant based diet (p= 0.01659 and p= 0.03982, respectively), no differences were detected according to sex and BMI. The ratio of total IgA/IgG leptin antibodies was also found elevated in omnivorous individuals (p=0.0094). In omnivore group, a negative correlation between the free fraction of IgA anti-leptin antibodies and glucose was found (r=-0.2928, p=0.0285), as well as the free fraction of IgG and body fat (r=-0.2668, p=0.0469). In plant-based group a positive correlation between IgG immune complexes fraction and body fat was found (r=0.3614, p=0.0421). **Conclusion:**IgA leptin reactive antibodies are modulated by different dietary patterns, possibly by the interaction between gut microbiota and different food antigens that possess molecular mimicry with leptin hormone, evidencing novel interactions between diet, the immune system and metabolism.

128. 648. **THE ROLE OF THE LIPID DROPLETS IN THE PATHOGENESIS OF LISTERIA MONOCYTOGENES**

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1. Fundação Oswaldo Cruz - FIOCRUZ

Background: *Listeria monocytogenes* (*L.m*) is a gram-positive pathogenic bacterium and the etiological agent of listeriosis. Although listeriosis usually results in mild gastroenteritis, severe infection with *L.m.* can result in more serious complications such as septicemia, meningitis, endocarditis or miscarriage, especially in at-risk populations (immunocompromised, elderly and pregnant women). Although it doe not cause the most cases of foodborne illness, listeriosis has attracted attention due to its high mortality rate in severe cases. Lipid droplets (LDs) are complex and dynamic organelles involved in the energy and lipid homeostasis of eukaryotic and prokaryotic cells. Numerous studies carried out in recent decades have shown that different pathogens target the LDs of the host, subverting them for both immune evasion and microbial proliferation. Recent results also indicate that LDs participate in the pro-inflammatory response. **Objective:** The objective of this study was to analyze the dynamics of LD accumulation and its involvement

in macrophage pathogenicity during *L.m*. infection. **Methods:** For it, bone marrow-derived macrophages were infected in vitro with *L.m.* (MOI 10) for 1 hour. LD biogenesis was assessed 1h, 6h and 24h after infection. **Results:** Our results shows that LD biogenesis depends on *L.m.* viability and virulence genes, in particular the activity of the pore-forming protein listeriolysin O (LLO). Pharmacological modulation of LD formation by inhibiting diacylglycerol O- acyltransferase 1 (DGAT1) and cytosolic phospholipase A2 (cPLA2) significantly reduced intracellular bacterial survival, impaired prostaglandin E2 (PGE2) synthesis and reduced IL-10 production. **Conclusion:** Finally, our data suggest that the participation of LDs contributes to *L.m.* intracellular survival and evasion in macrophages, suggesting that LDs contribute to the pathogenesis of *L.m.* infection.

129. 652. HIF-1-ALPHA INFLUENCES MITOPHAGY THROUGH BNIP3 AND PARKIN-DEPENTENT PATHWAYS IN INFLAMMATORY MACROPHAGES

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Mitophagy, an essential process for cellular quality control, eliminates defective mitochondria via PINK1/Parkin or non-canonical pathways, such as via BNIP3. However, the role of mitophagy in macrophages (macs) metabolism remains unclear. The hypoxia-inducible factor-1α (HIF-1α) induces the expression of BNIP3 and is crucial for the metabolic adaptation of inflammatory macs. Our aim is to determine the role of HIF-1α in mitophagy in inflammatory macrophages - LPS+interferon-γ (LPS/γ) macs. To this end, Western blot, Immunofluorescence, Seahorse and RNASeq assays were performed using bone marrow derived cells from Parkin knock-out and Hif-1a-deficient mice. First, we used a pharmacological autophagy inhibitor (Bafilomycin) to investigate the role of mitophagy in inflammatory macrophages. Bafilomycin treatment reduced glycolysis in LPS/γ macs. RNASeq data showed that BNIP3 was reduced in HIF-1α-deficient macs (HIF-1α$^{-/-}$), as well as BNIP3 protein levels, while LAMP1 and pDRP1 levels were increased. Additionally, increased colocalization of mitochondria and autophagosomes was observed in HIF-1α$^{-/-}$ LPS/γ macs. HIF-1α$^{-/-}$ macs displayed higher amounts of autophagosomes and lysosomes, with no changes in mitochondria mass, suggesting that mitophagy is inhibited. The mRNA expression of lysosomal Cathepsins was decreased in HIF-1α-deficient macs, indicating that an alteration in the lysosomal compartment may be affecting the degradation of mitochondria. We also determined the role of HIF-1α in mitophagy via different pathways. First, we examined the stabilization of HIF-1α in Parkin deficient-macs. The deletion of Parkin in LPS/γ macs resulted in reduced levels of HIF-1 α and BNIP3, suggesting that HIF-1α is involved in Parkin-mediated mitophagy. TNF and IL-6 levels were also reduced in Parkin-deficient macs, as also observed in HIF-1α$^{-/-}$ macrophages. Moreover, Parkin-deficient macs displayed increased bactericidal capacity together with increased production of reactive oxygen species. Together, these data indicates that HIF-1α regulates mitophagy through both canonical and non-canonical pathways in inflammatory macs, which may affect their metabolic adaptation and function.

130. 670. ASSESSMENT OF THE IMPACT OF MITOCHONDRIAL FUSION AS A KEY MECHANISM IN THE INDUCTION OF REGULATORY T LYMPHOCYTES MEDIATED BY MITOCHONDRIAL TRANSFER FROM UMBILICAL CORD MESENCHYMAL STEM CELLS TO NAIVE T-CD4+ LYMPHOCYTES

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Background: Mesenchymal Stromal/Stem Cells (MSCs) possess the ability to modulate the immune system. A mechanism employed for this purpose is mitochondrial transfer, a process that promotes the population of regulatory T cells (Treg). The enhance Treg population has been proposed as an alternative approach to treat autoimmune disease. However, the fate of these exogenous MSC-mitochondria once inside the target cell remains unknown. The mitochondrial integration, through mitochondrial fusion could be a plausible event that might be associated with Treg generation upon mitochondrial transfer. **Objectives:** We postulate that the generation of Treg population by mitochondrial transfer depends on mitochondrial fusion **Methods:** We use Umbilical Cord MSC (UC-MSCs) as mitochondrial donors for a population of naive T-CD4+ cells from healthy subjects. After the mitochondrial transfer, T-CD4 naïve were differentiated into Tregs using CD3, CD28, IL-2, and TGF-b1. After 72 hours we evaluated the expression of Treg markers indicators of their differentiation, proliferation, and molecules associated with their functional properties by flow cytometry and ELISA. Additionally, fusion was assessed by qRT-PCR. **Results:** We observed an increase on Treg cells that acquired new mitochondria. These observations were correlated with changes in mitochondrial dynamics on Treg cells that was associated with fusion events. *This might represent the potential mechanism by which mitochondria transfer induces Treg in vitro in naïve T-CD4 cells.*

131. 680. **STUDY OF THE IMMUNOMODULATORY EFFECT OF MITOCHONDRIA DERIVED METABOLICALLY REPROGRAMED MESENCHYMAL STEM/STROMAL CELLS TO MEMORY CD4-T CELLS.**

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Introduction: Metabolic reprogramming towards glycolytic metabolism of Mesenchymal Stem/stromal Cells (MSCs) have been shown to effectively suppress pro-inflammatory T cells. One of the restorative effects of MSCs is achieved through the transfer of their mitochondria (MT) towards T cells. Experimental evidence indicates T cell exhaustion in autoimmune diseases can restrict T cell activation through co-expression of immune checkpoints. However, it's unclear how the exhausted phenotype of memory CD4-T cells changes once it acquires MT from MSCs. Therefore, it is suggested that MT from MSCs to memory CD4-T cells could enhance the expression of various immune checkpoints, thus promoting a suppressive microenvironment. **Materials and Methods:** MSCs derived from umbilical cord (UC-MSCs) underwent metabolic reprogramming using 2-deoxyglucose (2-DOG), oligomycin and Tumor Necrosis Factor-alpha (TNF-α) plus interferon-gamma (IFN-y). Their mitochondria were stained with mitotracker green and isolated. In parallel, memory CD4-T cells from healthy donors were isolated from peripheral blood mononuclear cells (PBMCs) to perform artificial MT (Mitoception). These cells were sorted according to the acquisition or not of MT and activated for 4 days with CD3/CD28 and IL-2. Finally, flow cytometry was used to evaluate the expression of distinct immune checkpoints (CTLA-4, TIGIT, ICOS, PD-1, TIM-3, and LAG-3). **Results:** We observed a significantly enhanced expression of inhibitory receptors such as TIGIT and PD-1 in memory CD4-T cells with MT from UC-MSCs compared to those without. Moreover, we observed a significantly lower expression of T cell exhaustion surface markers such as TIM-3 and LAG-3 in memory CD4-T cells with MT from UC-MSCs. These differences did not depend on the metabolic status of UC-MSC. **Discussion:** This study represents a pioneering work in evaluating the effect of MT on inhibitory receptors of memory T-CD4 cells showing that MT increase the expression of surface markers associated with the suppressor capacity of T cells.

132. 694. **EVALUATION OF CD38 ACTIVITY DURING MONOCYTE MATURATION INTO MACROPHAGES AND ITS INFLUENCE ON CELL ACTIVATION BY NEUTROPHIL EXTRACELLULAR TRAPS (NETS)**

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Nicotinamide adenine dinucleotide (NAD) plays an essential role in cellular signaling and activation, acting as an enzyme substrate. Thus, NAD cellular levels must be tightly maintained and regulated. The molecule CD38 is the main enzyme responsible for regulating NAD levels and a major myeloid inflammatory marker, indicating its importance for the functioning of innate immune cells. Based on these findings, we hypothesized that there could be a correlation between CD38 activity and monocyte differentiation into macrophages, and with macrophage activation by neutrophil extracellular traps (NETs). NETs are molecular structures released by neutrophils upon contact with inflammatory mediators and infectious agents, characterized by chromatin exteriorization associated with cytoplasmic and granule proteins. We have observed that NETs activate macrophages through NF-kB pathway and induce higher production of reactive oxygen species (ROS) and inflammatory mediators. Here, we investigated the CD38 activity during monocyte maturation into macrophage, and NAD metabolism of macrophages upon interaction with NETs. NETs were obtained after neutrophil activation by IL-8, phytohemagglutinin, and inactivated SARS-CoV-2. Cells were exposed to purified NETs (centrifuged at 18k g) and, 24 h later, cell lysates and supernatants were collected for measuring intracellular NAD content and NADase activity by a fluorometric assay and production of inflammatory mediators by ELISA, respectively. We found that CD38 activity decreased during monocyte maturation into macrophages. Accordingly, the intracellular levels of NAD increased in macrophages in comparison to monocytes. In contrast, NADase activity was increased in macrophages stimulated by NETs, simultaneously to an enhanced pro- inflammatory response, as evaluated by production of IL-6 and TNF-alpha. These results suggest that NAD metabolism may regulate the 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.

133. 718. **IMPACT OF NUTRITIONAL STATUS ON INFLAMMATORY PROCESSES IN ADULTS IN PANAMA**

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Introduction: Excess weight in Panama exceeds 70% accompanied by the increase in the prevalence of chronic diseases, expressed through immunological alterations associated with increased secretion of proinflammatory cytokines. Predicting inflammation through body composition would reduce costs, time and injury and would contribute to the timely evaluation of the inflammatory process. General objective: To build a predictor of the inflammatory process according to the evaluation of body composition with the use of the secretion of inflammatory cytokines in adults from Panama. Methods: Pilot, analytical, cross-sectional study with a quantitative approach. 41 adults of 43% men and 57% women between 30 and 69 years old, from Panama and Western Panama, who signed consent through non-probabilistic evidence for convenience. People with gestational diabetes and inflammatory, autoimmune, intestinal, immunosuppressed diseases and with antibiotic or anti-inflammatory treatment were excluded. Interleukin 6 (IL6) and 1 (IL1), Tumor Necrosis Factor alpha (TNF alpha), and C-Reactive Protein (CRP) levels were evaluated by real-time mRNA expression. Nutritional status was assessed by: body mass index (BMI), abdominal circumference (WC) and % visceral fat (VF) by electrical bioimpedance (Inbody120). Chi2 and discriminant analysis were performed with confidence at 95%.The 79% were overweight (BMI □31 ±6) especially women (p=0.006), the mean and SD for WC and GV was 99.9 ±14.5 and 13 ±5.3, respectively. 76% and 72% presented CC and GV above normal. 67%, 3%, 36%, and 39%, respectively, presented values of TNF, PCR, IL1b and IL6b, indicating high inflammation. A relationship was found between IL6b with BMI (p<0.02). Discriminant analysis classified belonging to IL6b in 73%, to TNF in 60%, to IL1b in 70% and in 89% for PCR. Conclusions: The nutritional status serves to predict an increase in the immune status of people, being more reliable for PCR.

IMMUNOTHERAPY

134. 012. **HUMAN AMNIOTIC EPITHELIAL CELLS EXERT ANTI-CANCER EFFECTS THROUGH SECRETION OF IMMUNOMODULATORY SMALL EXTRACELLULAR VESICLES (SEV)**

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Background: Human amniotic epithelial cells (hAEC) are among placenta-derived cells with known immunomodulatory properties. We showed earlier that vaccination of mice with hAEC induced cross-protective immune responses and conferred effective protection in a mice model of colon cancer. Here, we extended our previous observation by examining the potential anti- cancer effects of hAEC-derived exosomes in murine models of colon (CT26), breast (4T1) and melanoma cancers (B16F10). **Methods:** hAEC were isolated from term placentas and characterized by immunophenotyping with a panel of stem cells markers. Different sets of experiments were performed to assess anti-cancer effects of hAEC including vaccination with live hAEC and hAEC lysate followed by orthotopic and heterotopic administration of tumor cells. Exosomes were derived from cultured hAEC (ADE) and their anti-proliferative and pro- apoptotic properties were performed by colorimetric and flow cytometric assays, respectively. Protective and therapeutic effects of ADE were also assessed in cancer models mentioned above. Enhancement of CTL responses in mice and percentage of CD4+ and CD8+ splenocytes following exosome injection was evaluated by Calcein AM (cAM) assay and flow cytometry. **Results:** Isolated hAEC showed high purity and expressed stem cells markers. Live hAEC conferred effective protection against colon cancer and melanoma but not in breast cancer in orthotopic administration. AEC induced strong cross-reactive antibody response to CT26 cells, but not against B16F10 cells. Heterotopic injection of tumor cells abolished the anti-cancer effect of hAEC vaccination. Mice vaccinated with hAEC lysate also showed no protection against melanoma or colon cancer. ADE induced apoptosis in CT26 cells and inhibited their proliferation. Co-administration of ADE with tumor cells substantially inhibited tumor development and increased CTL responses in vaccinated mice. ADE did not alter the frequency of spleen CD4+ and CD8+ cells. **Conclusion:** The results of the current study clearly demonstrated that although hAEC triggers cross-reactive humoral immune responses against tumor cells, these immune responses are not necessarily the major player in cancer preventive effects of AEC. Instead, it is the ADE that mediate most activity of AEC in prevention of cancer development. These findings highlighted the potential therapeutic application of ADE for cancer immunotherapy in the future.

135. 025. **EFFICACY OF THE THYMUS EXTRACT BIOMODULINA T IN CHILDREN WITH THYMUS HYPOPLASIA AND RECURRENT INFECTIONS**

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136. 030. **ENGINEERING CAR-T CELLS FOR DURABLE CONTROL AND CHECKPOINT BLOCKADE SYNERGY IN SOLID TUMORS**

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Background and Goals: CAR-T cells have shown remarkable clinical success in treating relapsed/refractory hematologic malignancies, and CAR-T adoptive cell therapy (ACT) is being avidly pursued for treatment of solid tumors. However, loss of full-range of T cell function over time (exhaustion), and impaired CAR-T persistence remain key challenges that limit the therapeutic potential in solid tumors. Hence, CAR- T engineering strategies that enhance survival and function are critical goals for therapy success. *Strategy, Results and Conclusions*: Using an immunocompetent preclinical model of CAR-T therapy of melanoma, here we show that ectopic expression of the pro-memory transcription factor Inhibitor of DANN binding 3 (Id3) in CAR T cells (Id3-CAR) augments solid tumor control *in vivo*. In addition to superior control of primary tumors, the Id3-CAR

T cells instilled greater long-term protection from tumor relapse in mice that cleared primary tumors compared to WT-CAR T cells. Surprisingly, augmented *in vivo* tumor control by the pro-memory transcription factor Id3 was associated with sustained cytoxicity and serial tumor killing *in vitro,* robust production of effector cytokines (IFN-y and TNF-a), as well as increased intratumoral localization compared to WT-CAR T cells. Id3-CAR T cells preferentially differentiated into TCF-1^{Hi} stem-like cells compared to WT-CAR T cells, with higher expression of memory-associated markers (such as Bcl-2, IL-7 and CD62L) and lower expression of markers of terminal differentiation (such as Tim3, granzyme B). Given the critical role of multipotent, self- renewing, stem-like TCF-1^{Hi} PD-1^{Int} exhausted cells in continuously feeding the transient effector T cell pool, persisting long-term and responding to PD-1 checkpoint blockade therapy in chronic viral infections and cancers alike, these data highlight Id3 and other such pro-memory transcription factors as attractive CAR engineering targets to bypass exhaustion in the TME, enhance PD-1 therapy outcomes and promote durable CAR T cell memory and protection from tumor relapse.

137. 035. **ISOLATION AND CHARACTERIZATION OF SINGLE DOMAIN ANTIBODIES AGAINST SARS-COV-2 RBD PROTEIN**

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In recent years, UDIBI has generated semisynthetic libraries of single-chain fragment variables (scFv), the development of these platforms has allowed us to obtain antibodies against the viruses: Chikungunya and SARS-CoV-2. Due to the success in the generation of these libraries and remarking the advantages and the superior properties of the sdAbs as high thermal stability, good tissue penetration, and low production costs. The present work had the purpose of generating and validating a single-domain semi-synthetic library. First, the construction of the single-domain semi-synthetic library was carried out from the assembly by PCR Overlap of fragments of a synthetic sdAb library with the CDRH3 natural fragments used in the assembly of ALTHEA Gold Libraries. Subsequently, the sdAbs specific against the RBD of SARS-CoV-2 were selected by means of screening in solution. SARS-CoV-2 RBD-specific sdAbs were identified through a monoclonal selection process and subsequent sequencing. It was possible to obtain 9 sdAbs with unique sequences capable of recognizing the RBD protein. The physicochemical characterization of the nine unique sequences by determination of SE-UPLC monomers was carried out and their molecular weight was determined by SDS PAGE.

138. 037. **TRANSFERON ORAL® ADMINISTRATION REDUCES SPECIFIC IGE LEVELS AND CELLULAR INFILTRATION IN NASAL-ASSOCIATED LYMPHOID TISSUE IN AN OVALBUMIN-INDUCED MURINE MODEL OF ALLERGIC RHINITIS**

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Transferon Oral® (TO) is a mixture of low molecular weight peptides with immunomodulatory properties. It was initially indicated for the treatment of viral infections such as herpes zoster in humans. Nevertheless, It was observed that TO improves the clinical signs of patients with allergic rhinitis (AR) too. AR is an IgE-mediated disease that affects the nasal mucosa, characterized by itching, sneezing and nasal congestion. There are only palliative treatments jfor AR, which affects 400 million patients worldwide. This study aimed to characterize the effect of TO in a murine model

of AR. To induce AR, female BALB/c mice (5-8 weeks old) were sensitized for 21 days by intraperitoneal injection of 200 µL of ovalbumin (OVA; 1 mg/mL) / aluminum hydroxide (1.3%) emulsion; then the animals were intranasally challenged (INC) with 10 µL of OVA (1 mg/mL) per nostril during 42 days. The establishment of AR was verified by quantitation of total and OVA-specific IgE serum levels. We evaluate the effect of TO (0.750 µg/200 µL) compared to Dexamethasone (0.125µg) in mice under INC treatment (n=12). We include as control healthy and RA mice. We evaluated the clinical status (Grimace scales); cell infiltration in NALT; cytokine and OVA-specific IgE levels by ELISA. The administration of TO in RA mice with INC statistically ($F=10.13$, $df(2,62)$, $P<0.002$) decreased the levels of anti-OVA IgE, and histopathological analyses revealed a lower infiltration of mast cells and eosinophils in the NALT compared to the untreated control group. The administration of dexamethasone resulted in a significant decrease in specific IgE levels at the conclusion of the treatment, but this occurred later compared to TO." This preliminary study suggests that Transferon Oral® may be an effective treatment for the management of allergic rhinitis, showing significant immunomodulatory effects in the studied murine model. Founding: CF-2023-G-836 CONAHCYT grant.

139. 041. **SIZE MATTERS; PLGA PARTICLE SIZE AS A CRITICAL MODULATOR OF ANTI-INFLAMMATORY RESPONSES AND IMMUNE TOLERANCE VIA ALPHA V BETA 3 MECHANO-SENSOR ENGAGEMENT**

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The increased incidence of auto-immune and inflammatory diseases demands the identification of immune-therapies modulating tolerance and resolution without the risk of systemic immune-suppression. Here we propose the use of biodegradable micro-particles as adjuvants; with the propensity to drive anti-inflammatory responses and antigen-specific tolerance. The Lavelle lab has focused on the importance of the physico-chemical properties of biomaterial-based adjuvants in the regulation of innate and adaptive immunity. Notably, particle size was found to be a critical factor that modulates dendritic cell activation and the subsequent activation and polarisation of T cell responses. We have identified that biodegradable poly (lactide-co-glycolide) (PLGA) particles within the narrow size window of 1-2 micrometre in diameter, drive potent secretion of IL-1Ra and IL-10 from key antigen presenting cells (APC's). Consequently, these anti-inflammatory APC's were proficient in priming and expanding a CD4+ regulatory T-cell (T-reg) population both in an *in-vitro* co-culture model and *in-vivo* studies. Investigations into the mechanism by which these particles enhance anti-inflammatory responses revealed a key role for the integrin; alpha v beta 3. Activation of this mechano-sensory integrin is hypothesised to be driven by changes in cell morphology and membrane tension induced by particles of the 1-2 micrometre range. Engagement of alpha v beta 3 increased downstream anti-inflammatory TGF-beta and signalling, initiating a positive feedback loop which promotes increased surface expression of alpha v beta 3 and the TGF-beta cell-membrane anchor; Glycoprotein A repetitions predominant (GARP). These findings demonstrate the therapeutic potential of biodegradable micro-particles of this size as an adjuvant to promote immunological tolerance in a manner independent of biologic agents or potent immunosuppressants. Furthermore these findings exhibit the importance of mechanical cues in driving immune responses, and how these can be harnessed in order to improve existing immune-therapies.

140. 042. **PADELIPORPHIN VTP REVERTS NSCLC ORTHOTOPIC TUMORS IN ANIMAL MODELS INTO HIGHLY RESPONSIVE TO IMMUNE CHECKPOINT INHIBITOR THERAPY**

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Background: Immune checkpoint inhibitors (ICIs) have shown promise as first-line treatments for NSCLC, yet patient survival rates remain lower than expected, highlighting the need to boost

antitumor immunity. Vascular targeted photodynamic therapy (VTP) with Padeliporfin (WST11, TOOKAD®), approved by the EMA for grade 1 localized prostate cancer and currently in Phase 3 trials for upper tract urinary cancer, triggers innate and adoptive antitumor responses in various animal tumor models. Concurrently, the NSCLC tumor microenvironment (TME) shows elevated immune suppressive signals like PD-1, PD-L1, and CTLA-4. We tested the hypothesis that combining VTP with clinically approved ICIs could enhance therapeutic efficacy in a mouse orthotopic model of NSCLC. Method: C57BL/6 mice bearing orthotopic LLC-Luc-mCherry tumors were subjected to Padeliporfin VTP with a relatively low light dose (60 mW/cm²) when bioluminescence measured by IVIS reached 107. Six doses of aCTLA4 (at -5 days VTP) and/or four doses of aPD1 (at +1 day VTP) were administered every 3 days until 10 days post-VTP. Tumor progression was monitored up to 90 days post-VTP using IVIS. H&E staining, mIHC, and MicroCT were performed on representative tumors. Results: Padeliporfin VTP induced up to 50% tumor necrosis at 3 days post-treatment, with 14% animal survival at day 90. Animals treated with aCTLA-4, aPD-1 and aPD-1 + aCTLA-4, reached 0%, 18% and 29% survival at day 90, respectively. Animals treated with VTP + aPD-1, VTP + aCTLA-4, and VTP + aCTLA-4 + aPD-1, were 33%, 40%, and 67% tumor free day 90, respectively. Micro CT analyses predicted treatment success as early as day 20 post-VTP application. Conclusion: VTP synchronized with ICIs combinations, effectively treats orthotopic NSCLC in mice. The safety of VTP and its impact on the immune landscape, will be tested in a Phase 1 clinical trial for peripheral lung cancer (NCT05918783).

141. 073. **A NOVEL PEPTIDE-LIGAND CONJUGATE IMMUNOTHERAPY TARGETING DENGUE VIRUS NON-STRUCTURAL PROTEIN 1 FOR TREATING MILD AND SEVERE INFECTIONS**

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Dengue virus (DENV) infections have surged dramatically, with reported cases rising from 505,430 in 2000 to 2,809,818 in 2022, highlighting the urgent need for effective treatments. Among the eleven structural and non-structural proteins of DENV, Non-structural protein 1 (NS1) stands out as a promising target due to its multifaceted role in modulating the immune response, inducing vascular leakage, and facilitating viral replication and assembly. Currently, monoclonal antibodies are the only therapeutics targeting NS1, but concerns about their cross-reactivity persist. Our study focuses on designing a novel Peptide Ligand Conjugate (PLC) as an alternative immunotherapeutic agent against NS1. This PLC aims to mediate the immune elimination of soluble NS1 and NS1-presenting DENV-infected host cells by leveraging pre-existing vaccine-induced immunity. Using High Throughput Virtual Screening (HTVS), QikProp analysis, and Molecular Dynamics studies, we identified three hits from Asinex Biodesigned Ligands out of 220,177 compounds that show strong binding affinity towards the monoclonal binding site of the NS1 protein. Following a rigorous analysis of physicochemical characteristics, antigenicity, allergenicity, and toxicity, we selected two peptides—the minimum epitopic regions of the Diphtheria and Tetanus toxins—as the peptide components of the PLCs. A non-cleavable, non-reactive oxime linker connected the ligand with the peptide through oxime and amide bonds. Given that the DPT vaccine is widely used in dengue-endemic countries and that antibody titers against the minimum epitopic regions of Diphtheria and Tetanus toxins persist lifelong in DPT-vaccinated individuals, the rationally designed PLCs are anticipated to bind NS1 through the ligands, triggering an immune response against NS1 by activating pre-existing DPT antibodies and memory cells. This orchestrated immune response is expected to destroy soluble NS1 and NS1-expressing DENV-infected cells, thereby reducing the severity of dengue hemorrhagic fever and overall DENV infection. Considering the growing need for therapeutics against DENV further investigation into this innovative immunotherapeutic strategy may provide a promising new approach for treating both mild and severe dengue infections.

142. 077. **ENHANCEMENT OF IMMUNE CHECKPOINT THERAPY THROUGH TARGETING MYELOID CELLS**

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Immune checkpoint inhibitors targeting PD-1,

PD-L1, and CTLA-4 have revolutionized the field of cancer therapy. These immunotherapies have shown great success in extending patients' survival, but only a limited effect is achieved in "cold tumors". It has been shown that immune suppression in the tumor microenvironment, caused by various populations of myeloid cells, directly impact the response to these therapies. Therefore, we propose redirecting the PD-L1 blockade to these non-tumor cells, which could improve the specificity of the interaction and promote the endocytosis of the inhibitory ligand, resulting in better outcomes compared to monotherapy. To this end, we generated bispecific antibodies based on nanobodies, targeting both PD-L1 and specific myeloid receptors. The antitumor effect was evaluated in murine models of colon carcinoma, and biodistribution was assessed by fluorescent imaging. We observed slower tumor growth and extended survival in mice treated with our construct under various treatment regimens compared to the control groups. 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.

143. 094. **PD-1 CHECKPOINT BLOCKADE RESTORES T-CELL EFFECTOR FUNCTIONS AND CONTROLS ACTIVE CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA MEXICANA**

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Cutaneous leishmaniasis (CL) is a neglected tropical disease caused by vector- borne protozoan parasites. *Leishmania mexicana* is the etiological agent of CL in Mexico, leading to a range of clinical outcomes from self-healing localized infections to chronic diffuse leishmaniasis (DCL). T cells from DCL patients exhibit functional impairment associated with an exhausted phenotype, characterized by the up- regulation of programmed death-1 (PD-1). In this study, we evaluated the effect of anti-PD-1 immunotherapy in a murine model with active cutaneous lesions caused by *L. mexicana*. Anti-PD-1 antibody (clone RMP1-14) was administered intraperitoneally to C57BL/6 mice every 3 days at a dose of 250 µg per mouse for a total of three doses starting on day 45 post-infection, followed by a 5-day resting period, and then five additional doses of 100 µg per mouse every three days. Our data showed that treatment with the anti-PD-1 blocking antibody controls the growth of footpad lesions throughout the follow-up period and significantly reduces parasite burden in both lesions and draining lymph nodes (dLNs). Furthermore, we observed that anti-PD-1 therapy restores the antigen-specific production of IFN-γ, TNF-α, and IL-2 cytokines in dLNs cells. Blocking PD-1 signaling also led to an increase in $CD4^+IFN\text{-}\gamma^+$ and $CD8^+IFN\text{-}\gamma^+$ producing cells, as well as $CD8^+CD107a^+$ cytotoxic cells. Similarly, we observed an increase in proliferative $CD4^+$ $Ki\text{-}67^+$ and $CD8^+$ $Ki\text{-}67^+$ cells in the anti-PD-1-treated mice. Finally, we demonstrated that anti-PD-1 treatment primarily promotes the expansion of $CD4^+CXCR5^+$ and $CD8^+CXCR5^+$ progenitor cells with an exhausted phenotype, which retain their effector functions. Overall, these results demonstrate that blocking the PD-1/PD-L1 inhibitory pathway revitalizes T-cell effector functions, promoting the control of active *L. mexicana* infection. These findings highlight the therapeutic potential of anti-PD-1 antibodies for the treatment of DCL, a condition for which there is currently no effective treatment. Funding: Conahcyt Fronteras: 6682 y PAPIIT IG200924.

144. 110. **OPTIMIZATION AND CHARACTERIZATION OF GALECTIN-8 PRODUCTION IN MAMMALIAN CELL SYSTEMS**

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Background: Galectins are a family of mammalian lectins that play crucial roles in the immune response. To produce recombinant galectin-8 (Gal-8) for biotherapeutic applications, we expressed the human Gal-8 M isoform in HEK293T cells using lentiviral transduction. Since galectins are secreted via an unconventional pathway, a heterologous signal peptide was employed to enhance Gal-8 secretion. **Objectives**: Since the human Gal-8 sequence contains putative *N*-glycosylation sites at both carbohydrate recognition do-

mains (CRDs), we aimed to generate and characterize different recombinant Gal-8 mutants in the HEK293T cells. **Methods**: Gal-8 mutants were generated by substituting asparagine (Asn) with alanine (Ala) at site 52 in the *N*- CRD (Gal-8N52) and at site 255 in the *C*- CRD (Gal-8N255). The double mutant Gal-8N52/255, containing point mutations at both sites, was also generated. The proteins were purified from cell culture supernatants using lactose-affinity chromatography. Sugar residues on the different recombinant mutants were identified by a lectin blot assay employing an array of biotinylated plant lectins. Finally, Gal-8 lectin activity was then determined via hemagglutination assay. **Results**: Binding of Concanavalin-A (ConA), Maackia amurensis-I (MAL-I), Ulex europaeus agglutinin (UEA-I), and Wheat Germ agglutinin (WGA) to both Gal-8 wild-type (Wt) and Gal-8N52 was observed. However, Gal-8N255 was recognized exclusively by the WGA lectin. In all cases, recognition occurred at the band with the highest apparent molecular weight, confirming that the purified isoforms correspond to different Gal-8 glycoforms, with the *C*-CRD being the most extensively *N*-glycosylated. The various glycoforms of Gal-8 exhibited similar hemagglutination activity, ranging between 0.5 - 1 µM, suggesting that glycosylation does not significantly affect its activity. **Conclusions**: We successfully expressed and purified different Gal-8 glycoforms and confirmed their activity through hemagglutination assays. Future functional testing of these Gal-8 glycoforms will be conducted in T cells, dendritic cells, and endothelial cells to critically assess their biotherapeutic potential.

145. 114. **BACK TO BASICS: BACTERIA-BASED IMMUNOTHERAPY USING SALMONELLA FOR MELANOMA TREATMENT**

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Among skin cancers, melanoma is the most lethal type, with a low 5-year overall survival rate in metastatic patients. Additionally, it shows an increasing incidence in young population. Bacteria-based immunotherapies offer unique advantages in the fight against melanoma, since this tumor is highly immunogenic. Unlike conventional cancer treatments, which have limited tumor specificity, bacteria as *Salmonella* selectively colonize tumors and kill cancer cells, through diverse mechanisms. In this work we aim to dissect the changes in melanoma cell proteins after *Salmonella* LVR01 infection through mass spectrometry. Proteomic analysis comparing non-infected (control) vs infected melanoma cells identified 2269 eukaryotic proteins, being 2173 in control samples and 2067 in infected cells. Furthermore, 1971 proteins were identified in both conditions, being 202 and 96 proteins exclusively identified in control or infected cells, respectively. Relative abundance analyses showed 32 proteins overrepresented in infected samples (enriched proteins, EP) and 64 proteins overrepresented in the control group (repressed proteins, RP). For EP, there was an increase in biological processes corresponding to response to stimuli and cell proliferation and growth, while enrichment in molecular functions as nucleic acid binding and electron transfer activity, among others. Conversely, for the RP we observed enrichment in biological processes related to regulation and cellular component organization and localization. Regarding molecular functions, we found enrichment in protein binding and hydrolase activity. Three separate pathways of interaction were overrepresented, corresponding to mRNA processing, keratinization and energy production. Interestingly, the latter was overrepresented in both EP and RP datasets, suggesting that *Salmonella* infection might influence cancer metabolism. Candidates were then contrasted with existing literature, including their use as prognostic markers in diverse types of cancer, and protein expression changes were further confirmed by RT-qPCR. In this way, we obtained new candidate molecules that could explain the antitumor activity exhibited by *Salmonella* LVR01.

146. 115. **VIRAL VECTOR VACCINATION IMPROVES ADOPTIVE T-CELL THERAPY AGAINST SOLID TUMOURS**

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Adoptive T-cell therapy (ACT) consists of systemic infusion of cytotoxic tumour specific T cells aiming to control neoplasias. However, ACT still has very limited efficacy in humans. Cancer vaccines are promising immunotherapies to improve ACT, but the mechanisms underlying this combination are still poorly understood. To identify novel therapeutic approaches to improve ACT against solid tumours and to elucidate key factors responsible for immunotherapy efficacy, we have combined ACT with the chimpanzee adenovirus ChAdOx1 and poxvirus MVA vaccines encoding for specific tumour antigens. Strikingly, mice treated with the combination therapy showed a markedly higher tumour regression as compared to monotherapy controls. This efficacy was correlated with a superior tumour infiltration and expansion of tumour-reactive T cell populations in lymphoid organs. Surviving mice were rechallenged and monitored for 250 days and revealed that specific memory T cells populate lymph nodes, spleen and bone marrow. Interestingly, inflammation alone during ACT was sufficient to enhance T cell expansion and tumour control when followed by antigen specific boosting. This synergy was abrogated when T cell trafficking to the lymph nodes was impaired. Importantly, we found that ChAdOx1 and MVA vaccination induces high levels of CXCL10 and subsequent blockade of its receptor, CXCR3, completely abrogated vaccine-mediated T cell expansion and tumour control, suggesting a crucial role for CXCR3 signalling in the synergy between T cell therapy and cancer vaccines. In conclusion, ChAdOx1 and MVA vaccination improves ACT against solid tumours by expanding transferred T cells that have long-lasting memory in a CXCR3-dependent manner.

147. 116. **CLINICAL TRANSLATABLE STRATEGY FOR ACHIEVING PROSTATE TUMOR-FREE OUTCOMES THROUGH ANTI-GALECTIN-3-BASED CHEMOTHERAPY COMBINED WITH A VACCINE AS AN EFFECTIVE IMMUNOTHERAPY PROTOCOL.**

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Patients with metastatic castration-resistant prostate cancer (mCRPC) continue to die due to ineffective or resistant treatments. At this stage, the primary proposed therapies are chemotherapy with taxane derivatives or immunotherapies, although the latter is less emphasized due to tumor evasion mechanisms. Interestingly, it has been observed that the use of Docetaxel (DTX) enhances the effectiveness of certain immunotherapies in combination therapies. Recently, we demonstrated that gene expression analysis of prostate cancer (PCa) cells revealed that treatment with DTX reduces Galectin-3 (Gal- 3) expression in both human and murine PCa cell lines, whether sensitive or resistant to DTX. More importantly, downregulation of Gal-3 was confirmed in clinical samples from mCRPC patients treated with taxane-based chemotherapy (RNA-seq GSE147493 and GSE1111777). We also showed that Gal-3 is one of the factors contributing to the failure of anti-CaP immunotherapies, influencing several molecular and cellular processes. To investigate this, we established a preclinical murine PCa model using s.c. transplanted TRAMP-C1 cell lines (TC1) in syngeneic C57BL-6 mice. Following primary tumor resection, mice were treated with a vaccine composed of bone-marrow-derived dendritic cells loaded with downregulated Gal-3 TC1 cell lysates, using the Sipuleucel-T vaccine as a model. Thus, we demonstrated that systemic downregulation of Gal-3 with non- cytotoxic doses of DTX prior vaccination was enough to elicit an effective anti-tumor immunoresponse and protect mice against tumor recurrence. In pursuit of more clinically translatable options, we optimize our vaccine by using only tumor cell lysates to utilize endogenous dendritic cells as antigen-presenting cells showing highly protection against tumor recurrence or metastasis. This is an encouraging result for all PCa patients, indicating that Gal-3 inhibition with taxane-based chemotherapy could be an effective component of a combined strategy to enhance the efficacy of immunotherapies in patients with mCRPC, alongside a straightforward vaccine that could be easily implemented in clinical settings.

148. 117. **NEW IMMUNOTHERAPY FOR CANCER: INHIBITION OF GALECTIN-1 IN T LYMPHOCYTES**

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We previously demonstrated that endogenous galectin-1 in lymphocytes plays a critical role in regulating immunity in cancer. In this study, we further explore this biological process and develop an intervention strategy based on this understanding. First, we demonstrated that lymphocytes purified from tumors and tumor-draining lymph nodes express increased levels of galectin-1 transcripts upon in vivo activation, compared to the same type of cells from mice without tumors. These results highlight a transcriptional mechanism through which tumors modulate this galectin in their local immune microenvironment. This prompted us to design and assess a new gene control strategy specifically targeting galectin-1 expression in T lymphocytes. To do this, we use interfering RNA against galectin-1. To control its biodistribution in the body, we linked this shRNA to an RNA aptamer specific for 4-1BB (CD137, a member of the tumor necrosis factor receptor superfamily induced upon T cell activation). We demonstrate that this molecular tool effectively and significantly decreases galectin-1 in activated T lymphocytes in vivo. Subsequently, we evaluated the impact of this molecular tool during an OVA-peptide immunization. We demonstrate that inhibition of galectin-1 in T lymphocytes enhances the specific cytotoxic response induced by immunization. Appropriate statistical studies supported the relevance of our experiences. These results lay the foundations for an original immunotherapy for cancer.

149. 118. **LOW DOSE OF A COMBINED FORMULATION OF ADJUVANTS IMPROVES IMMUNE ACTIVATION IN CANCER VACCINES**

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Adjuvants play a crucial role in regulating the magnitude and type of immune responses triggered during immunizations. This study focuses on two immunostimulatory molecules (CpG and Poly-U) capable of generating a TH1 response, a beneficial profile for cancer vaccines. While vaccine development has historically been inspired by adjuvant knowledge in infectious diseases, it's important to recognize that cancer and infection involve distinct cellular and molecular requirements. Therefore, this study aims to determine if the use of adjuvants in cancer requires specific calibration. The study evaluated CpG and Poly-U individually and in combination, utilizing the currently used doses of 10 nmol and 100 µg per injection, respectively. A 16-fold lower dose was also compared to assess its impact on lymph node immunophenotypes, in vivo cytotoxic function, and tumor growth. The results revealed that individual use of adjuvants at the currently used doses yields sub-optimal functional responses. In contrast, administering lower doses of both adjuvants in combination created a more favorable microenvironment for immunization in the lymph nodes. This was evidenced by a mild expansion of T lymphocytes and increased infiltration of cross-presenting dendritic cells, along with a lower proportion of Treg and myeloid-derived suppressor cells. Additionally, the lower doses of combined adjuvants resulted in greater specific in vivo cytotoxicity in an OVA immunization model (40+6 versus 18+7, $p<0.01$, t- test). Furthermore, prior immunization with lower doses of combined adjuvants provided better anti-tumor protection against an OVA-expressing melanoma. Interestingly, while the currently established doses of adjuvants led to higher T lymphocyte numbers in lymph nodes, it was associated with a more suppressive pattern and a lower functional response. The findings challenge current concepts for cancer immunotherapy, suggesting that better effector function is consistently associated with moderate T cell expansion and a non- suppressive microenvironment, rather than high immune expansion.

150. 137. **DEVELOPMENT OF POTENTIAL LPS-NEUTRALIZING AGENTS DERIVED**

FROM THE HUMAN SCAVENGER RECEPTOR CD6

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CD6 is a lymphocyte-specific receptor involved in lymphocyte activation/differentiation as well as in recognition of bacterial-associated molecular patterns. It is a trans-membrane glycoprotein with an ectodomain composed of three tandem scavenger receptor cysteine-rich (SRCR) domains. CD6-knockout mice studies revealed its relevance in the immune response to bacterial infections, partially due to its ectodomain's ability to recognize lipopolysaccharide (LPS). Accordingly, the infusion of the soluble recombinant ectodomain of human CD6 showed prophylactic and therapeutic effects in mouse models of bacterial infections. The bacterial-binding properties of CD6 have been mapped to homologous 11-mer peptide sequences present in its SRCR domains. Herein, we analyzed the LPS-neutralizing activity of potential immunotherapeutics derived from the human CD6 ectodomain. Synthetic peptides were designed and commercially-obtained with high purity: three 11-mer peptides corresponding to each SRCR domains (pCD6.D1, pCD6.D2 and pCD6.D3) and a larger 35-mer peptide composed of those sequences aligned in tandem (pCD6.D1-D2-D3). Additionally, recombinant CD6 subunits corresponding to the SRCR domains CD6.D1 and CD6.D3 were produced and purified from transiently transfected HEK293T cells, either with (rCD6.D1-hFc and rCD6.D3-hFc) and without (rCD6.D1 and rCD6.D3) the fusion of a C-terminal human Fc fragment (hFc) to favor dimerization. The optimization of domain production allowed the generation of highly pure rCD6.D1 and rCD6.D3 with yields of 1.0 and 0.7 mg per 100 mL of culture, respectively; increasing to 2.4 and 2.2 mg per 100 mL for rCD6.D1-hFc and rCD6.D3-hFc, respectively, after hFc addition. The capacity of these agents to bind and neutralize bacterial components was evaluated using immunoassays and in-vitro cell cultures stimulated with LPS, with nitric oxide secretion serving as an indicator of LPS-neutralizing activity. Preliminary results allowed us to compare the performance of these molecules at the micromolar level, enabling the identification of the most promising components for developing novel immunotherapeutics to treat severe bacterial infections.

151. 143. **CONTROL OF RHABDOID TUMORS BY POLY(I:C) IS MEDIATED BY A GLOBAL REMODELING OF THE TUMOR MICROENVIRONMENT**

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Rhabdoid tumors (RTs) are aggressive pediatric tumors that historically have been considered poor candidates for immunotherapy due to their genetic similarity to self. However, we have demonstrated that RTs possess epigenetic mechanisms of tumor immunogenicity and are highly infiltrated by clonally expanded CD8+ T and myeloid cells. These tumors contain activated CD8+ T cell subpopulations expressing druggable inhibitory checkpoints, such as PD-1, Tim-3, and LAG-3. Although blockade of the PD-1/PDL-1 pathway induces regression of established RTs in mice, some tumors escape or resist treatment, demanding improved immunotherapeutic strategies. Deep immune characterization of the human and mouse RT microenvironment (TME) revealed that tumor-associated macrophages (TAMs) are the most abundant cell subpopulation. Depletion of macrophages in CD64-hDTR mice delayed tumor growth, highlighting their negative impact. Given the high expression of TLR3 in myeloid cells infiltrating RT samples, we treated RT-bearing mice with intratumoral administration of poly(I:C) (PIC), a synthetic dsRNA and TLR3 ligand, resulting in a significant delay in tumor growth. To elucidate the underlying mechanism, we analyzed the effects of PIC on TME using FACS, scRNAseq, and immunofluorescence. Our results suggest that PIC treatment reduced protumoral macrophages, increased tumor infiltration by neutrophils and CD8+ T cells, and induced

iNOS expression in peritumoral macrophages. iNOS inhibition abrogated neutrophil and CD8+ T cell recruitment. PIC also activated TLR3-expressing conventional dendritic cells (cDC1), accumulating in draining lymph nodes. Additionally, PIC promoted progenitor exhausted CD8+ T cells (TPEX) in the TME. Given the association of TPEX cells with anti-PD-1 response, we combined PIC with anti-PD-1, resulting in complete tumor rejection and full memory against tumor rechallenge. Our findings indicate that modulation of the TME by PIC overcomes RT resistance to anti-PD-1 treatment, representing a promising immunotherapy approach for clinical translation.

152. 167. **LOCAL ADMINISTRATION OF THE INMUNOMODULATORY STAPHYLOCOCCAL PROTEIN A AS A NOVEL BIOTHERAPEUTIC STRATEGY FOR THE TREATMENT OF CUTANEOUS INFECTIONS CAUSED BY METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS**

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Staphylococcus aureus is the leading cause of skin and soft tissue infections (SSTIs). Staphylococcal protein A (SpA) promotes beneficial immune responses in the skin by the early recruitment of neutrophils, the modulation of their lifespan and the induction of proper abscesses formation, processes that are vital for bacterial eradication and wound healing. Therefore, SpA might be a promising candidate for co-adjuvant immunomodulatory therapies against *S. aureus* SSTIs. This study was aimed at evaluating the efficacy of the local administration of heat-killed *Lactococcus lactis* SpA as immunomodulator and inductor of wound healing in an established cutaneous MRSA infection in mice. Healthy mice were intradermally inoculated with either 10^8 CFU of *L. lactis* SpA or *L. lactis* CV (control vector), or PBS. Histological analysis at 6 days post-inoculation revealed a significant increase in the number of mature mast cells in the dermis of mice inoculated with *L. lactis* SpA compared with the control groups ($p<0.05$, parametric ANOVA and Tukey's post-test), with no evidence of skin damage in either group. Subsequently, mice were subcutaneously inoculated with 10^7 CFU of *S. aureus* strain USA300LAC (day 0), followed by intradermal administration of either *L. lactis* SpA, *L. lactis* CV 24 hours later. A significant reduction in the skin lesion size was observed overtime in mice treated with *L. lactis* SpA which was not observed in the control group ($p<0.001$; paired one-way ANOVA and Tukey's post-test). Histopathological studies of the skin at day 7 demonstrated that mice treated with *L. lactis* SpA had organized abscess structures with defined fibrous walls and localized polymorphonuclear infiltration surrounding the bacterial community. In contrast, the lesions in the control group were more extensive, with dispersed bacterial communities. These findings suggest the feasibility of using *L. lactis* SpA as a locally delivered immune modulator in the skin during *S. aureus* SSTI.

153. 170. **SALMONELLA LVR01 INDUCES DUAL INNATE IMMUNE MEMORY RESPONSES IN TUMOR MODELS**

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Innate immune memory operates in two modes: trained immunity, which amplifies cellular responsiveness, and tolerance, which reduces the immune response to later challenges. Agents such as beta-glucan, *Leishmania*, BCG, and LPS are known to trigger one of these effects. BCG, an established immunotherapy for bladder cancer, uses trained immunity to enhance anti- tumor adaptive responses. *Salmonella* has also shown potential in cancer treatment, generating strong

but short-lived anti-tumor immune responses. This study investigates whether attenuated *Salmonella* LVR01 can induce trained immunity and how this influences anti-tumor activity. *In vivo* stimulation of bone marrow cells with *Salmonella* LVR01 was performed, followed by a secondary stimulus to evaluate trained immunity through cytokine production. The impact of *Salmonella* LVR01 on tumor growth and survival was examined in mouse models, with tumors implanted after bacterial administration. In vitro assays were conducted to measure cytokine production in mouse monocytes following stimulation with *Salmonella*. LVR01 led to an increased cytokine response in bone marrow cells, consistent with trained immunity. This enhanced response was associated with slower tumor growth and improved survival in treated mice. However, *in vitro* studies showed that stimulation of monocytes with *Salmonella* resulted in reduced cytokine production, indicating immune tolerance. These dual effects may explain the temporary benefits of *Salmonella*-based cancer therapies, underscoring the need for further research to refine this approach.

154. 182. **CARBOHYDRATES ON THE CRYPTOCOCCUS SPP. SURFACE AS TARGETS IN THE BIOENGINEERING OF CHIMERIC ANTIGEN RECEPTOR**

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Cryptococcus neoformans is an opportunistic fungal pathogen affecting immunocompromised individuals. Annually, approximately one million cases of meningoencephalitis are reported, resulting in about 625,000 deaths worldwide. Current therapies for cryptococcosis often lead to adverse side effects and antifungal drug resistance is a challenge, and cell therapy using chimeric antigen receptors (CARs) can overcome the issues involved in the treatment of cryptococcosis. Two CAR constructs targeting (Glucuronoxylomannan) GXM were developed, 2H1-GXMR-CAR and 18B7-GXMR-CAR. Three novel CAR constructs specific to chitin, Chitin1-CAR and Chitin2-CAR, or galactoxylomannan (GXMGal), GXMGalR-CAR were generated. All of these CAR constructs contain CD8 molecule as hinge/transmembrane domain, CD137 as co-stimulatory portion, and CD3zeta as activator domain. 2H1-GXMR-CAR and 18B7-GXMR-CAR expressed by Jurkat cells induced high levels of IL-2 production and increased CD69 expression, and 18B7-GXMR-CAR Jurkat cells showed highest levels of activator markers. Chitin-CAR and GXMGalR-CAR lentiviral titers reached levels at least 3.73x10^7TU/mL. Jurkat cells modified with Chitin1-CAR or Chitin2-CAR (MOI3), were not able to induce T cell activation in the presence *of C. neoformans*, as demonstrated by the levels of IL-2 production and CD69 expression. In contrast, GALXMR-CAR Jurkat cells had high levels of IL-2 production and CD69 expression in the absence of the target indicating tonic signaling, and the incubation of GALXMR-CAR Jurkat cells and *C. neoformans* did not change significantly the activator markers. Therefore, 2H1- GXMR-CAR and 18B7-GXMR-CAR were chosen in the modification of peripheral blood mononuclear cells (PBMCs), resulting in 45.8% and 23.01% of positive cells, respectively. These modified cells were co-cultured with *C. neoformans* and high levels of IFN-gamma were found by ELISA. Taken together, these data demonstrate that GXM is the major carbohydrate that can be targeted by CAR for the T cell redirection to attack *Cryptococcus neoformans*. This study opens perspectives in the evaluation of fungicide activity of GXMR-CAR T cells against cryptococcosis.

155. 191. **VACCIMEL, AN ALLOGENEIC MELANOMA VACCINE, EFFECTIVELY TRIGGERS T-CELL IMMUNE RESPONSES AGAINST NEOANTIGENS AND ALLOANTIGENS, AS WELL AS AGAINST TUMOR-ASSOCIATED ANTIGENS**

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VACCIMEL is a therapeutic cancer vaccine composed of four irradiated allogeneic human melanoma cell lines expressing multiple tumor-associated antigens (TAA). We demonstrated that vaccination in the adjuvant setting significantly prolongs the distant-metastasis-free survival of melanoma patients and that T-cells reactive to TAA and to patients' private neoantigens increased during treatment. However, immunization to vaccine antigens arising from VACCIMEL's somatic mutations and polymorphisms remains unexplored. To investigate this, we performed whole-exome sequencing of paired tumor and germline samples from four vaccinated patients and the vaccine cells. VACCIMEL variants were identified by comparing the exomes with MuTect2. Epitope candidates were predicted using MuPeXI. The candidates were prioritized based on mRNA expression in the vaccine, predicted peptide-MHC presentation, and stability. Then, immune responses were tested using IFNy-ELISpot assays on vaccinated patients' PBMC samples. An average of 9481 non-synonymous coding variants were detected in VACCIMEL when compared to germline exomes from the patients. Between 0.05 and 0.2% of the variants were also found in the tumors of three vaccinated patients, and one patient with a high tumor mutational burden (TMB) shared 19.5%. T-cell reactivity assessment showed that patients mounted diverse responses against peptides not present in their tumors, comprising alloantigens and neoantigens. Additionally, the patient with high TMB was immunized against public neoantigens shared between her tumor and VACCIMEL. Notably, T-cells targeting the patient's tumor antigens comprising neoantigens and TAAs were more frequent than those targeting VACCIMEL-exclusive antigens. Finally, antigen expression in VACCIMEL and immune responses were correlated. These results indicate that the immune system simultaneously responds to numerous antigens, either vaccinal or private, demonstrating that immunization to off-target epitopes was not detrimental to immunity against relevant neoantigens and TAA.

156. 215. **ANNEXIN A1 SIGNALING PATHWAY AS A POTENTIAL THERAPEUTIC TARGET FOR TEGUMENTARY LEISHMANIASIS.**

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Cutaneous leishmaniasis is a parasitic neglected tropical disease that causes slow-healing lesions that can lead to long-lasting scars. Social and self-stigma have been known to influence the quality of life and well-being of patients, urging the search for more efficient therapies that could limit lesion development. Annexin A1 (AnxA1) is a glucocorticoid-inducible protein known for its anti-inflammatory and pro-resolving properties. AnxA1 actions can be mimicked by the administration of its N-terminal domain with 26 amino acids termed Ac2-26 peptide. Recent studies have demonstrated a therapeutic potential for Ac2-26 in infectious diseases, including cutaneous leishmaniasis. We have shown that the lack of endogenous AnxA1 is associated with susceptibility during *Leishmania amazonensis* infection. Moreover, treatment of *L. amazonensis*-infected AnxA1 KO mice with Ac2-26 increases the production of anti-inflammatory cytokines and improves pathogen clearance. Therefore, we aimed to further explore the protective effects of Ac2-26 in cutaneous leishmaniasis. Systemic treatment of WT mice with Ac2-26, by i.p injection, increases the numbers of activated T cells, improving the clearance of the parasite. To evaluate whether local treatment with Ac2-26 could potentiate these effects, we developed a topical formulation and administered it to WT mice. Mice treated topically presented diminished lesions, parasite burden and IFN-γ production when compared with i.p. treated mice. Interestingly, we found lower numbers of Th2 cells and $CD4^+$ $Arginase1^+$ T cells and increased Tregs in topically-treated mice compared to i.p.-treated mice. These results could signify that local treatment of *L. amazonensis* lesions with Ac2-26, rather than systemic administration, is a more efficient way to balance the effector responses with the regulatory responses, preventing parasite replication while controlling the damage caused by the exacerbated inflammation. Our findings suggest topical treatment with Ac2-26 may be an effective treatment approach to localized cutaneous lesions, helping reduce the social impact of the disease in patients.

157. 217. **HARNESSING GLYCOBIOMARKERS: A GLYCO-IMMUNE SIGNATURE (GIS) FOR IMPROVED IMMUNOTHERAPY RESPONSE PREDICTION**

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Background: Immunotherapies (IT) are effective antitumoral therapies, yet predicting patient outcomes remains challenging. While biomarkers like PD-L1, tumor mutational burden, and microsatellite instability (MSI) are relevant in the clinics, their standalone efficacy is limited. Notably, aberrant glycosylation is linked to tumor development, but its role in predicting IT outcomes is underexplored. This study investigates how glyco-immune gene expression could identify patterns that predict IT responses. **Methods:** TCGA data was obtained from GDC, and IT-treated patients' data from PRJEB23709 and GEO (GSE200997). Deconvolution of tumor microenvironment (TME) was performed using MIXTURE with LM22 signature. Gene signatures, CMS, and MSI classification were obtained from MSigDB or literature. All analyses were performed using R software v4.4.2. **Results:** Clustering TCGA-SKCM (melanoma) samples using glyco-immune genes revealed two glycoclusters (GCs). GC2 was immunoactive and showed better survival. From these profiles, we developed a Glyco-Immune Signature (GIS) and validated it using biopsies from IT-treated patients, where responders were enriched in GC2 patients. Our signature positively correlated with response signatures, and negatively with resistance signatures**,** and proportional-hazard models indicated that its use could improve discrimination between high and low-risk patients. In colorectal cancer, we found associations between MSI status and response to IT, with MSI-H patients and responders showing higher GIS scores. High GIS-scoring patients presented a "hot" TME, higher scores of immune-related signatures and correlated with CMS1 classification. Interestingly, ~40% of MSI-L/MSS patients showed high GIS scores. Single-cell data revealed that high GIS-scoring patients were enriched in effector CD8 T cells, and reduced infiltration of Tregs, T CD4 annexin-1+ and Th17 cells. These results are being evaluated in mouse models. **Conclusion:** Our GIS could serve as a surrogate marker for current biomarkers associated with IT response, aiding in treatment selection for patients, including those MSI-L/MSS patients who lack approved IT options but show promising glycosignature profiles.

158. 264. SMALL EXTRACELLULAR VESICLES FROM DIFFERENT SUBSETS OF T REGULATORY CELLS REVEAL CELL DEATH-RELATED PROTEINS AS CARGO HIGHLIGHTING CYTOTOXICITY AS SUPPRESSION MECHANISM

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T regulatory cells (Tregs) act as modulators of immunity. One of their suppressive mechanisms is the release of small extracellular vesicles (sEV). Tregs can be classified based on their origin: thymic (nTregs) and induced (iTregs) Tregs. However, it is unknown whether their origin determines their mechanism of action, including the properties of their sEV. Thus, in this study we investigated the composition of nTregs- and iTregs-derived sEV and their suppressive function *in vitro*. nTregs and naïve CD4+ T-cells were purified using magnetic beads. nTregs were cultured for 72h and iTregs were induced with IL-2 and TGF-β alone (iTregs) or with retinoic acid (RATregs). sEV were purified using ultracentrifugation and IZON columns and were characterized by size and concentration using the Nanoparticle-tracking analysis (NTA) equipment. Tandem-mass spectrometry (MS/MS) was performed to identify sEV protein content. Suppression assays were performed by polyclonally activating splenocytes for 72h in the presence of sEV obtained from the three types of Tregs. Cell phenotype, proliferation, cytokine release and apoptosis were evaluated by flow cytometry and ELISA. Our results indicate that sEV obtained from the three-types of Tregs share similar characteristics such as particle's number (10^8 part/mL) and size (~150 nm). Suppression of T cell proliferation was sEV-dose-dependent, observing that iTregs-derived sEV are less effective on inhibition that sEV obtained from nTregs or RATregs. With respect to cytokine production, sEV from RATregs induce the release of IFN-γ and IL-17. Moreover, sEV proteomic revealed an enrichment for cell death-related proteins which was confirmed by differential induction of apoptosis and necrosis on activated splenocytes. In this

regard, sEV from iTregs and RATregs resulted more apoptotic than nTregs, which stimulated necrosis. In conclusion, these results reveal a novel possible mechanism for Tregs-derived sEV, highlighting the role of cell death-related proteins that may be causing apoptosis and necrosis of target cells.

159. 274. **EXPLORING IMMUNE BIOMARKERS AND SURVIVAL ASSOCIATIONS IN HER2-NEGATIVE BREAST CANCER PATIENTS UNDERGOING COMBINATION THERAPY WITH IMMUNE CHECKPOINT INHIBITORS**

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The integration of immune checkpoint inhibitors (ICBs) in cancer treatment has opened new avenues for personalized therapy, particularly in challenging subtypes like HER2-negative breast cancer. This study investigates immune signatures and clinical outcomes in 26 women aged ≥18 years with advanced HER2-negative breast cancer, treated with a combination of bevacizumab (anti-VEGF) and durvalumab (anti-PD-L1) administered every four weeks. Using mass cytometry (CyTOF), we analyzed CD45+ immune cells at weeks 0, 4, 8 and 28. At week 0, non-responders (NR) exhibited significantly elevated subsets of Mo PDL1+, Treg, and Naive B cells. Survival analysis revealed that increased Mo PDL1+ (p= 0.009) and Treg Ki 67- (p= 0.046) at week 0 were associated with poorer survival , whereas higher levels of activated CD4+ T cells correlated with improved survival (p= 0.038). This pattern persisted through weeks 4 and 28, indicating that activated CD4+ T cells may be critical for long-term outcomes. To extend our findings, we conducted a comparative analysis using single-cell RNA sequencing (scRNAseq) data from tumor tissues. These data, from a published study (Zhao et al., 2021), focused on patients treated with paclitaxel combined with anti-PD-L1 therapy. This cohort, chosen for its relevance to our immunotherapeutic regimen, allowed us to explore the tumor microenvironment. Our analysis identified differentially expressed Treg subtypes in the tumor tissue of responders compared to non-responders, suggesting a potential parallel between peripheral blood and tumor immune landscapes. These findings highlight the complexity of immune responses in HER2-negative breast cancer, emphasizing the significance of both pro-tumor and anti-tumor immune populations identified at baseline. The integration of blood and tumor data is crucial for a comprehensive understanding of these mechanisms, guiding the development of personalized and more effective therapeutic strategies in this challenging breast cancer subtype.

160. 326. **EFFECT OF TARGETED DEMETHYLATION OF THE FOXP3 GENE BY CRISPR-TET1 TO GENERATE A REGULATORY-LIKE PHENOTYPE IN HUMAN LYMPHOCYTES**

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Background: Regulatory T lymphocytes (Tregs) are a key CD4+ T subpopulation that suppresses exacerbated immune responses. Their development and function require surface proteins, such as CTLA-4 and PD-1, cytokines such as TGF-β and the transcription factor Foxp3. Stable expression of Foxp3 in Tregs depends on demethylation of the TSDR/CNS2 intronic region of the Foxp3 gene. **Objective**: This project aimed to develop an epigenetic editing protocol with CRISPR- TET1 to generate human lymphocytes with a regulatory T phenotype. **Methods**: Plas-

mid (pSpdCas9-TET1-sgTSDRhuman-EGFP) was constructed including the TET1 enzyme, involved in demethylation by catalyzing the conversion of 5-mC to 5-hmC, an inactive form of Cas9 (dCas9), working in conjunction with a guide RNA to direct the system to the TSDR of the human FOXP3 gene, and a fluorescence marker (EGFP) for cell identification and isolation by flow cytometry and flow assisted cell-sorting. The vector was electroporated in the Jurkat human lymphocyte cell line. Control cells were electroporated with a plasmid comprising the same elements except for a catalytically-inactive form of TET1. The viability and efficiency of transfection were assessed by flow cytometry. EGFP-positive transfected cells were isolated by FACS. Finally, the expression of genes associated with the Treg phenotype and effector T lymphocytes (Th) was analyzed by quantitative real-time RT-PCR. Statistical significance was analyzed by Student's T-tests or Analysis of Variance (ANOVA). **Results**: Our results show that targeted demethylation of the TSDR region by CRISPR- TET1 increases the expression of FOXP3 and suppression markers such as CTLA-4, PD-1 and TGF-beta while decreasing the expression of markers associated with Th2 and Th17 lymphocytes, such as STAT6 and ROR-gamma-T in Jurkat cells.
Conclusion: The use of CRISPR-TET1 to demethylate the TSDR region can modulate the expression of genes related to Treg stability and suppressor capacity in human lymphocytes, suggesting possible applications against inflammatory and autoimmune diseases.

161. 335. **NON-CANONICAL PROTEOME DERIVED FROM TRANSPOSABLE ELEMENTS IS A SOURCE OF FUNCTIONAL PROTEIN ISOFORMS AND CANCER NEOANTIGENS**

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Recent advances in ribosome profiling and mass spectrometry-based proteomics have expanded our understanding of the proteome by uncovering non-canonical proteins. Among these, transposable elements (TEs), which constitute 45% of the human genome and are repetitive sequences dispersed throughout it, play a significant role. TEs can serve as hosts for open reading frames in non-genic regions or participate in unannotated splicing events with protein-coding exons. This study explores the role of splicing between exons and TEs as a source of unannotated functional isoforms and tumor-specific neoantigens. Using transcriptome assembly, ribosome profiling, and mass spectrometry we showed that exonized TEs can be efficiently translated, resulting in a population of low-abundance isoforms that are generally shorter but stable. We characterize their subcellular localization and demonstrate that their functions can diverge from those of canonical isoforms. While many TE-derived isoforms are specific to individual samples, some are shared across different individuals. Moreover, a subgroup of these isoforms is recurrent in lung cancer patients but absent in healthy tissues. Importantly, we provide evidence using immunopeptidomics that these unannotated isoforms encode HLA-I presented peptides, which are immunogenic and can be recognized by infiltrating CD8 T cells in lung tumors and tumor-invaded draining lymph nodes. Different TE families display varying capacities to encode stable protein isoforms or, on the contrary, short-lived polypeptides that enter the HLA-I processing pathway. Our findings underscore the evolving nature of TE-derived proteins, which, while exploring new functions, may produce unstable and rapidly degraded products, giving rise to HLA-presented, recurrent, immunogenic, and tumor- specific antigens in cancer patients. Overall, our study emphasizes the clinical relevance of TE-derived antigens as promising targets for cancer immunotherapy and offers insights into the role of non-canonical splicing in

TEs during protein evolution.

162. 344. **HEMATOPOIETIC STEM CELL TRANSPLANTATION INCREASES SERUM LEVELS OF IL-10 IN PATIENTS WITH SYSTEMIC SCLEROSIS**

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by immune dysregulation, vascular damage, and fibrosis of the skin and internal organs. Autologous Hematopoietic Stem Cell Transplantation (AHSCT) has been used as treatment for patients with severe and progressive forms of the disease. The therapeutic effect of AHSCT involves depletion of the autoreactive T and B cells, renewal of the lymphocyte repertoire, and improvement of immunoregulatory mechanisms. Herein, we aimed to evaluate serum inflammation and immunoregulation-related molecules before and after AHSCT. Methods: In this retrospective study, serum samples from 14 patients were evaluated for TNF-α, IFN-α, IFN-γ, CCL2, CCL3, IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, IL-21, IL-31, and IL-33 using a multiplex assay. Data were initially evaluated for normal distribution by the Shapiro- Wilk test. Way-ANOVA or Kruskal-Wallis tests followed by Tukey's or Dunn's multiple comparisons tests were used to analyze differences between means of three or more groups. Results: Comparing the levels of each serum marker at different time points before and after transplantation, there was a decrease in IFN-γ levels at 12 months ($p<0.05$), while CCL-2 and IL-8 decreased at 24 months ($p<0.05$). Serum levels of TNF-α, IL-13, IL-31, and IL-33 remained unchanged after transplantation in SSc patients. Among all serum cytokines analyzed, only the anti-inflammatory molecule IL-10 showed an increase at 12 ($p<0.05$) and 24 ($p<0.01$) months compared to baseline; there was also an increase from 12 to 24 months ($P<0.01$) after AHSCT. Conclusion: Notably, serum levels of key proinflammatory cytokines, such as IFN-γ, CCL-2, and IL-8, showed significant reductions post-transplantation. This suggests that AHSCT can modulate the inflammatory milieu, contributing to the clinical improvements observed in these patients. Additionally, the significant increase in serum levels of IL-10, a crucial immunoregulatory cytokine, post-transplantation, highlights the important mechanisms of action of AHSCT in the immunoregulation pathway.

163. 345. **IMMUNE RECONSTITUTION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IS ASSOCIATED WITH CLINICAL OUTCOMES IN PATIENTS WITH SYSTEMIC SCLEROSIS**

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164. 347. **UNRAVELLING THE ROLE OF IFN-GAMMA IN SALMONELLA LVR01-MEDIATED ANTI-TUMOR EFFECT**

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Salmonella is a facultative anaerobic pathogen that has been widely studied as an immunotherapy given its anti-tumor effects. However, the molecular and cellular mechanisms underlying its effects are not yet fully understood. Our previous research demonstrated that one intratumoral injection of an attenuated *Salmonella* Typhimurium strain (*aro*C-, LVR01) slows primary tumor growth

and extends survival in melanoma B16F1-bearing mice (Vola et al., 2018). Additionally, LVR01 neoadjuvant treatment enhances surgical outcome by reducing metastases, leading to a tumor-free survival rate above 50% (Mónaco & Plata et al., 2022). In the early stages of the disease, we have reported that tumor growth is controlled by innate effectors, such as NK cells and macrophages, but not by CD8+ T cells (Mónaco et al., 2022; Mónaco & Plata et al., 2022). In this work, we studied the role of IFN-gamma, involved in the function and activation of these cells, in *Salmonella* mediated anti-tumor effect with the help of IFN-gamma knock-out (ko) mice. We found that IFN-gamma is not essential in *Salmonella*-mediated effect on the primary tumor, as LVR01 had the same potential, determined as tumor growth and survival, in wt and IFN ko mice. Accordingly, no clear differences were found in tumor-infiltrating immune cells between wt and IFN ko animals. Moreover, we could not find IFN-gamma in sera of LVR01- treated mice (for up to 8 days). However, we found that IFN-gamma is crucial for LVR01-mediated control of metastasis development after primary tumor excision (Log Rank Test, ****p<0,0001). In this regard, the roles of NK cells, CD8+T cells and macrophages still remain to be elucidated in the melanoma metastatic model. All things considered, these results highlight the different immune mechanisms underlying the control of primary tumor and metastasis elicited by *Salmonella*.

165. 349. **MULTI-APPROACH ANALYSIS IDENTIFIES TUMOR-DERIVED 4EBP1 AND CCL4 AS POTENTIAL SERUM BIOMARKERS FOR IMMUNOTHERAPY RESPONSE**

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Multi-approach Analysis Identifies Tumor-Derived 4EBP1 and CCL4 as Potential Serum Biomarkers for Immunotherapy Response

Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment, particularly in melanoma. However, not all patients respond to these therapies, highlighting the need for predictive biomarkers. We previously demonstrated that elevated blood levels of p4EBP1 and CCL4 before ICI initiation correlate with poor treatment response in melanoma patients. Here we investigated our hypothesis that tumors producing higher amounts of these proteins could release them into circulation.

We conducted a comprehensive multi-modal analysis of melanoma tumor microenvironment. Our approach integrated three distinct datasets: (1) single-cell RNA sequencing (scRNAseq) data from melanoma patients, irrespective of treatment status; (2) public bulk-RNA sequencing data from pre-treatment melanoma tumors with known clinical outcomes; and (3) scRNAseq data from tumor-infiltrating immune cells in melanoma patients with documented ICI responses. Our analysis revealed that 4EBP1 is expressed by various cell types, predominantly tumor cells, endothelial cells, and macrophages, while CCL4 is primarily expressed by CD8+T cells and NK cells. Although bulk expression levels of 4EBP1 and CCL4 didn't significantly differ between non-responders (NR) and responders (R) at baseline, we observed notable differences in immune cell populations. NR patients exhibited higher frequencies of CCL4-expressing CD8+T cells (p=0.0074) and monocytes/macrophages (p=0.0068) compared to R patients. Similarly, EIF4EBP1-expressing monocytes/macrophages were more frequent in NR patients (p=0.0003). Importantly, NR patients showed overall higher frequencies of CD8+T cells (p=0.005) and monocytes/macrophages (p=0.007) within their tumors. These findings suggest that the elevated blood levels of 4EBP1 and CCL4 observed in NR patients before ICI therapy may originate from their tumors, with monocytes/macrophages primarily contributing to 4EBP1 levels and CD8+T cells to CCL4 levels. Our study provides new insights into the tumor immune microenvironment associated with ICI response and highlights the potential of 4EBP1 and CCL4 as predictive biomarkers.

166. 353 **ASSESSMENT OF THE ANTITUMOR POTENTIAL OF CD8+ T CELLS INDUCED BY TRYPANOSOMA CRUZI INFECTION AGAINST THE B16-F10 CELL LINE**

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Pathogen-specific T cells have been reported to recognize neoepitopes via cross-reactivity, leading to antitumor responses. Previous experiments for our group showed that both acute infection with *Trypanosoma cruzi* and infection treated with Benznidazole delay tumor growth in C57BL/6 mice challenged with B16-F10 cells. This study aimed to evaluate the contribution of $CD8^+$ T cell cross-reactivity generated in response to infection to this effect. Adult female C57BL/6 mice were randomly divided into three groups: G1 received 500 *T. cruzi* trypomastigotes (Tulahuen strain) intraperitoneally 15 days before tumor challenge; G2 and G3 received adoptive transfer of $2x10^6$ $CD8^+$ T cells, isolated from spleens of infected and healthy mice, respectively, intravenously on the day of tumor challenge after positive selection with CD8a magnetic microbeads. All mice were inoculated subcutaneously with 200.000 B16-F10 cells. Significant differences in tumor volume were observed between groups (*Kruskal-Wallis* test, $p<0.05$; G1 vs. G3 and G2 vs. G3, $p<0.01$ (day 22), *multiple comparisons test*). Interestingly, in G2, a population of $CD8^+$ T cells specific for $Tskb20^+$, an immunodominant epitope of *T. cruzi*, proliferated after adoptive transfer. However, targeting dendritic cells with an anti-CLEC9A antibody conjugated to Tksb20 and combined with the Poly(I:C) adjuvant (G4), showed no changes in tumor progression compared to immunization with the adjuvant alone (G5), a non- specific anti-CLEC9A antibody conjugated to Tskb20 (G6), or a control group without any immunization (G7). These results suggest that targeting a single epitope may not be sufficient to elicit a potent antitumor response, though it is evident that the antitumor effects of *T. cruzi* infection are at least partially mediated by $CD8^+$ T cells.

167. 367. INCREASING THE POTENCY OF CD40 AGONISTIC NANOBODIES BY GENERATING RECOMBINANT TRIMERIC AND BIPARATOPIC CONSTRUCTS

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Agonistic antibodies that activate immune-stimulating receptors hold promising therapeutic potential. Particularly, CD40 agonistic antibodies strongly activate antigen presenting cells (APC), with excellent performance as vaccine adjuvants or as APC-targeting constructs with antigens, promoting robust cytotoxic T cell and B cell responses, and also as cancer immunotherapy drugs for their capacity to turn “cold” tumors into “hot” ones. Most anti-CD40 antibodies used in preclinical and clinical settings are conventional whole IgGs, which rely on FcγRIIb binding for crosslinking and, therefore, activity. Our group has produced novel mouse CD40 agonistic nanobodies derived from *Llama glama.* These small, easily produced recombinant proteins become potent agonists when fused to T4 fibritin-“foldon” domain for trimerization (Nb-foldon, prokaryotic expression) or to mouse IgG1 Fc for FcγRIIb binding (Nb-mFc, eukaryotic expression). However, Nb-foldon are only active in vitro, potentially due to poor stability and short half-life, while Nb-mFc are effective both in vitro and in vivo but could be optimized for lower doses. In this study, we propose modifications to both constructs in order to improve their performance. Nb-foldon were modified by introducing cysteine residues that promote interchain pairing and by adding a short terminal peptide for neonatal receptor binding, while Nb-mFc were converted into biparatopic antibodies by combining two of our nanobodies that bind non-overlapping epitopes on CD40, which, based on previous work, may enhance agonistic activity. After successful expression of these new constructs, they are currently being tested for activity, and we hope the results will be valuable not only for developing CD40 agonists but also for other immune-stimulating receptors.

168. 370. DEVELOPMENT OF THE FAB VERSION OF THE MONOCLONAL NIVOLUMAB (ANTI-PD-1) AS AN IMMUNOBIOLOGICAL TOOL FOR PROSPECTING NEW ANTITUMOR STRATEGIES

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Programmed Cell Death Protein 1 (PD-1) is a crucial immune checkpoint receptor in the tumor microenvironment. Monoclonal blocking antibodies for this checkpoint were developed to restore exhausted T cells’ ability to eliminate tumors. However, pharmaceutical companies outside Latin America produce most of these commercial antibodies in mammalian cells or hybridomas. The microbial expression system in *Escherichia coli*

proposes a faster and lower- cost alternative for producing these molecules. In this scenario and in the context of improving public health and offering national biopharmaceuticals, this project aimed to develop and characterize the Fab version of the monoclonal Nivolumab (anti-PD-1). The synthetic pFab Nivolumab construction was transformed into *E. coli* standard strain BL21(DE3) for expression, followed by the soluble fraction protein A affinity chromatography to anti-PD-1 purification. The purification yield was 672,84 ug/L and analysis by SDS-PAGE of purified protein exhibited a single band at ~25 kDa, suggesting high purity. The antibody functionality was first analyzed by indirect ELISA, evaluating that the least amount of Fab needed to obtain 50% of the PD-1 antigen blockade response (EC50) is ~1018 nM. Functional analysis to evaluate its ability to modulate immune responses is ongoing. Our partial results demonstrate the successful expression and purification of the Fab anti-PD-1, if confirmed its ability to modulate immune responses, this molecule raises as a potential antitumor therapeutic tool.

169. 379. **GENERATION OF HUMAN MACROPHAGES DERIVED FROM CORD BLOOD HEMATOPOIETIC STEM CELLS FOR APPLICATION IN CANCER IMMUNOTHERAPY**

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Background: Advances in the field of onco-immunology have led to the comprehension of the crucial role of macrophages in the tumor microenvironment. Macrophages' abilities to infiltrate the dense tumor matrix, to promote phagocytosis, to present antigens and to secrete pro-inflammatory cytokines licensed these cells as promising for cancer immunotherapy. However, sources for obtaining macrophages are limited due to their low proliferative capacity. **Objectives:** Our main aim was to generate functional macrophages from umbilical cord blood $CD34^+$ cells (HSC) directing their application to cancer immunotherapy. **Methods**: Cryopreserved HSC were expanded *in vitro* and differentiated into macrophages (maccd34) using two distinct protocols: protocol 1 = SCF, GM-CSF, TNF-alpha + M-CSF; protocol 2 = GM-CSF, M-CSF, IL-3 + M-CSF. Maccd34 were than evaluated for morphology, phenotypic profile, phagocytosis capabilities and cytokine production. **Results:** By flow cytometry, about 85% and 54% of $CD14^+CD64^+$ maccd34 were obtained using RPMI and STEMSPAN culture media respectively for protocol 1. Those cells presented a $CD34^{neg}CD1c^{neg}HLA\text{-}DR^{high}CD163^{low}CD16^+CD86^{low}$ phenotype and showed a typical morphology of macrophage. In protocol 2 about 75% and 36.4% of $CD14^+CD64^+$ maccd34 were obtained using RPMI and STEMSPAN media, respectively, and cells presented a $CD34^{neg}CD1c^{low}HLA\text{-}DR^{high}CD163^{neg}CD16^{high}CD86^+$ phenotype. During differentiation, cells showed about 8,8X (RPMI) / 4,6X (STEMSPAN) fold expansion at day 5. Polarization with IL-4 induced up-regulation of CD64, HLA-DR, CD86 and CD1c molecules. For functional assays, maccd34 efficiently phagocyted phrodo zymosan microbeads and produce TNF-alpha and IL-1beta in response to lipopolysaccharide activation. In addition, maccd34 from protocol 2 showed a reduced ability of phagocytosis of SKBR3 tumor cells and a higher percentage for Nalm6 tumor cell phagocytosis compared to blood monocytes. **Conclusion:** Differentiated maccd34 showed phenotypic and functional similarities with classical monocyte-derived macrophages. Our ongoing steps include the generation and differentiation of maccd34 expressing chimeric antigen receptors for the amplification of their functionalities against tumors.

170. 386. **STUDY OF NANOVENOMS FORMED BY CROTOXIN ISOLATED FROM CROTALUS DURISSUS TERRIFICUS VENOM ADSORBED TO SILICA NANOPARTICLES**

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In Argentina, *Crotalus durissus terrificus* (*C.d.t.*) is one of the species of greater medical importance since its venom (V) contains mainly crotoxin (CTX), **a potent toxin** responsible for its high lethality. The generation of antivenoms (AVs) is an expensive task, involving immunization plans generated in animals specifically for this purpose. Nanoparticles (NPs) are currently being studied due to their potential therapeutic use. Thus, the possibility of using NPs transporting V toxins, would allow to obtain a novel complex (nanovenom-NV-) for the production of AVs. Therefore, in this work we proposed to study the physicochemical and immunomodulatory activity NVs, formed by CTX and silica nanoparticles (SiNPs) of 150 nm diameter with both net charges (+/-). We isolate CTX from the *C.d.t.* V by FPLC and adsorb it to both SiNPs synthesized by Stöber method. These complexes (NVs) were analyzed by TEM and by SDS-PAGE (14%) to assure the correct adsorption. For cytotoxicity analysis, MTT assay was performed on THP-1 cells exposed to different treatments for 48h. For the immunomodulatory activity, proinflammatory (IL-1β) and anti-inflammatory (IL-10 and TGF-β) cytokines were measured in culture supernatants by ELISA. The statistical analyses performed were ANOVA and Dunnett´s multiple comparisons test. TEM and SDS-PAGE analysis showed a correct adsorption of CTX on both types of SiNPs. No significant differences between the NVs, CTX and the whole V were seen in MTT assays. IL-1β shows a greater concentration in cell culture treated with both types of SiNPs. No significant differences were observed within the treatments for anti-inflammatory cytokines. When THP-1 cells were activated with LPS we observed significant differences for TGF- β, showing that NVs generates a greater concentration than the SiNPs. These results allow us to continue studying the NVs for their possible use as adjuvants in the production of AVs.

171. 405. VENETOCLAX RESISTANT CELLS FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) ARE SENSITIVE TO ANTI-CD20 MONOCLONAL ANTIBODIES

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Background: The treatment of CLL patients with the BCL-2 inhibitor, venetoclax (ven) has demonstrated efficacy, but the emergence of resistant cells is a current complication. Although the combination of ven with anti-CD20 monoclonal antibodies (mAbs) is approved, the evidence of the effect of these mAbs on Ven-resistant cells is still scarce (doi:10.1182/bloodadvances.2019000180, doi: 1080/14737140.2023.2288899). **Objective:** To compare the effect of anti-CD20 mAbs on ven resistant and control CLL cells. **Methods:** Control and Ven-resistant CLL cells were employed as targets to evaluate the mechanisms of action of CD20 mAbs Rituxumab (Rx) or Obinutuzumab (Obz): direct cell death (DCD), complement dependent cytotoxicity (CDC), antibody dependent cell- mediated cytotoxicity (ADCC) and phagocytosis (ADCP). We generated ven-resistant cells by culturing peripheral blood mononuclear cells from nine unrelated CLL patients, with anti-CD3 antibody for 72h, with DMSO/Ven the last 24h (doi:10.3324/haematol.2018.188680). Then, viable CLL cells were purified and employed as targets. Statistical analyses were made using Wilcoxon's signed rank test, Friedman test followed by Dunn's multiple comparison test. **Results:** Ven-resistant cells from our model express lower CD20 levels compared to control ones (**p<0.01, n=8). CD20 mAbs induced DCD in control and Ven-resistant cells (**p<0.01), being Ven- resistant cells more susceptible to Rx than control ones (#p<0.05). Within ven-resistant cells, Obz was superior to Rx to induce DCD (#p<0.05), and Obz+Ven was superior to Rx+Ven (#p<0.05) or Ven alone (###p<0.001) (n=7). None of the anti-CD20 mAb induced CDC. For ADCC, only Obz generated CLL cytotoxicity and NK cell degranulation (*p<0.05), but these effects were reduced for ven-resistant cells (##p <0.01) (n=7). Rx and Obz favored ADCP (**p<0.01, n=9) without significant differences between the targets. **Conclusions:** Our in vitro results indicate that, despite the lower CD20 levels on ven- resistant cells, they are sensitive to Rx and Obz, being Obz superior to eliminate them by inducing higher DCD and ADCC.

172. 407. BIOENGINEERING MANNAN-SPECIFIC CAR-NK CELLS FOR ENHANCED

ANTIFUNGAL IMMUNITY AGAINST INVASIVE CANDIDIASIS

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Background: Invasive candidiasis is a severe infection primarily caused by *Candida albicans*. The World Health Organization classifies *Candida* infections as a critical priority for developing new therapeutic approaches, due to the rising of antifungal resistance. The *Candida* cell wall, mainly composed of virulence-associated carbohydrates, plays a key role in immune evasion. Natural killer (NK) cells are crucial for early defense, releasing cytotoxic granules and cytokines that promote T-cell differentiation. However, *Candida* spp. can evade these responses through morphological switching. Chimeric antigen receptors (CARs), synthetic receptors designed to redirect immune cells against specific targets, are a promising approach for treating invasive fungal infections and have been a focus of study in our group. **Objectives:** To bioengineer T and NK cell lines (Jurkat and NK-92) expressing a *Candida*-specific CAR (Ca-CAR) to target *Candida* species *in vitro* and *in vivo*. **Methods:** A second-generation CAR (Ca-CAR) targeting the *Candida* cell wall was developed and expressed in Jurkat and NK-92 cells via lentiviral transduction. GFP^+ cells were enriched by fluorescence-activated cell sorting (FACS). Screening for Ca-CAR-Jurkat cell activation in the presence of various *Candida* species was performed by measuring IL-2 levels. Ca-CAR-NK-92 cell activation and their effector activity against *C. albicans* were evaluated *in vitro* and in NSG mice. Glycan microarray analysis was performed to identify the Ca-CAR ligand. **Results:** Ca-CAR-Jurkat cells produced high IL-2 levels in response to *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. auris*, but not against other fungi. Ca-CAR-NK-92 cells showed increased IFN-γ and CD107a expression, significantly damaging *C. albicans in vitro*. *In vivo*, Ca-CAR-NK-92 cells reduced fungal burden in the kidneys of infected mice. Glycan microarray analysis confirmed Ca-CAR specificity for *Candida* mannan. **Conclusion:** This study provided proof-of-concept of a CAR that can effectively target *Candida* species and enhance the antifungal activity of NK cells against *C. albicans*.

173. 408. **EVALUATION OF THE IMPACT OF INHIBITING THE TGF-BETA PATHWAY IN CD8 LYMPHOCYTES AND THE TUMOR MICROENVIRONMENT ON THE ANTI-TUMOR RESPONSE**

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Transforming Growth Factor beta (TGF-β) promotes the progression of different types of cancer, including melanoma. In advanced stages, TGF-β inhibits the function of various immune cells types, such as dendritic cells, macrophages, natural killer cells, and CD4 and CD8 lymphocyte, creating a permissive microenvironment for tumor growth. Understanding the molecular mechanisms underlying the effects induced by the signaling of this cytokine in different cell populations will enable us to identify molecular targets for cancer treatment. The objective of our study was to evaluate the therapeutic effects of inhibiting the activity of molecular components of the TGF-β pathway in CD8 lymphocytes and

the tumor microenvironment. Using a murine melanoma model, we characterized the impact of inhibiting TGF-β signaling via the peritumoral injection of chemical inhibitors. Additionally, we evaluated the adoptive transfer of CD8 lymphocytes genetically modified by CRISPR Cas9 system to delete a specific molecular component of the TGF-β pathway. Analysis of tumor growth, tumor weight, and flow cytometry were performed using Student's t-test or ANOVA to determine statistical significance. Chemical inhibition of TGFBR1 in the tumor showed great therapeutic potential by promoting better tumor growth control, increasing IFNγ expression in CD8 lymphocytes, enhancing cytotoxic activity in NKT cells, and decreasing PD1 expression in CD4 lymphocytes. Local inhibition of SMAD3, the adoptive transfer of TGFBR2-deficient CD8 T cells, and combined therapy with inhibitors of TGFBRI and SMAD3 all showed favorable effects on tumor growth control. Altogether, these results suggest that inhibition of TGF-β signaling in CD8 cells and within the tumor microenvironment shows promise as a therapeutic strategy for melanoma treatment.

174. 420. **BONE MARROW-DERIVED APC VACCINE ENHANCED BY SILDENAFIL CITRATE BOOSTS IMMUNOGENICITY IN A MURINE MAMMARY ADENOCARCINOMA MODEL**

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Cellular therapies using antigen-presenting cells (APCs) derived from bone marrow show promise but face efficacy limitations. Drug repurposing, such as sildenafil citrate, is being explored to enhance immune responses and antitumor efficacy. This preliminary study assessed how in vitro modulation of APCs with sildenafil could optimize cellular vaccines in breast tumor models. APCs were differentiated from mouse bone marrow over 6 days and stimulated with cytokine cocktail and sildenafil for 24 hours. The EO771 tumor cell line was inoculated into the mammary glands of 8-week-old female C57BL/6J mice (ethical protocol: 6051-1/2022). After 2 weeks of tumor growth, 6 groups were established: chemotherapy alone (D), combined chemotherapy with modulated APC vaccine (CDV), combined chemotherapy with systemic sildenafil and modulated APC vaccine (CDVS), and control groups; (n=3). Animals were treated for 3 weeks with chemotherapy (doxorubicin, 3 mg/kg, twice weekly), APC vaccine (weekly), and sildenafil (10 mg/kg daily). Tumors were analyzed by flow cytometry for $CD40^+CD80^+$ APCs, $CD4^+$ and $CD8^+$ T lymphocytes, and tumor aggressiveness. Statistical significance was determined using the Student's t-test, with $p<0.05$ considered significant. The vaccine comprised 62% $CD11c^+$MHC II^+ APCs. The CDVS group tumors showed significantly increased $CD4^+$ and $CD8^+$ T lymphocyte infiltration compared to the negative control group. This approach also enhanced $CD40^+CD80^+$ APCs infiltration and reduced $CD24^+Ki67^+$ tumor populations but increased $CD24^+Ki67^+STAT3^+$ cells, indicating agressive phenotype selection. Tumor weight in the CDVS group was 133 mg, significantly lower than in the negative control group (1142 mg) and non-modulated vaccine groups (693 mg). These preliminary results suggest that multimodal approaches with modulated APC vaccines enhance tumor immunogenicity reinforcing the potential of combined therapies. Future studies should explore the molecular mechanisms of sildenafil in APC capabilities and refine strategies to avoid selecting resistant tumor subpopulations.

175. 441. **PHARMACOVIGILANCE OF A REGIONAL GAMMA GLOBULIN: REGISTRY YEARS 2015-2023 IN THE DIVISION OF ALLERGY AND CLINICAL IMMUNOLOGY OF THE HOSPITAL DE NIÑOS DE LA SANTISIMA TRINIDAD, CORDOBA, ARGENTINA.**

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INTRODUCTION: Endovenous immunoglobulin (IV) therapy is the treatment of choice in some primary immunodeficiencies (PID). The development of gamma globulin from regional donors constitutes an improvement in the quality of the product according to WHO recommendations. OBJECTIVES: To evaluate the presence of Adverse Drug Reactions (ADRs) in a group of patients treated with Immunoglobulin G EV UNCR in patients with PID at the Hospital de Niños de la Santísima Trinidad. MATERIAL AND METHODS:

We analyzed infusions of ImmunoglobulinG EV UNC in children and adults from 01/01/2015 to 31/12/2023 at the Hospital with a diagnosis of Antibody Deficiency. Hospitalizations was in the day hospital sector with infusion pump and prior administration of cetirizine, vital signs and thermal controls were performed before, during and at the end of the procedure. In the group of patients there was an episode of headache that subsided with a decrease in the drip rate and NSAIDs, an episode of arterial hypertension associated with the infusion that required a change of brand, and in another patient a reaction of hypotension, vomiting and diarrhea that forced suspension of the infusion and a change of brand. All 3 events were in different patients and received previous cetirizine. The ADRs analyzed refer to the time of infusion. CONCLUSIONS: Good tolerance was evidenced in the pediatric population studied, demonstrating a satisfactory safety profile during the procedures.

176. 451. **FUNCTIONALIZED NANOPARTICLES IMMUNOTHERAPY FOR TARGETED TRAIL MRNA DELIVERY TO METASTATIC NICHES**

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Purpose: Immunotherapy has advanced cancer treatment by enhancing antitumor immuneresponseswhileminimizingoff-targeteffects[1]. However,treatingmetastasesandsolidtumorsremains challenging due to the lack of specific cell surface targets, which hinders tumorelimination without harming healthy tissues. Claudin 6 (CLDN6) [2]and the tumor necrosisfactor-related apoptosis-inducing ligand (TRAIL) are promising targets for immunotherapy [3].This study developed a lipid nanoparticle (LNP) platform functionalized with anti-CLDN6antibodies to deliver TRAIL mRNA within the tumor microenvironment, promoting selectivetumor cell death. **Methods:** TRAIL mRNA was encapsulated in LNPs composed of ionizablelipids,cholesterol,andlipid-PEG-Maleimideviamicrofluidicmixing. TheLNPswerefunctionalizedwithanti-Claudin6antibodies(TRAIL-LNP-Fuc)usingthiolationandcharacterized by dynamic light scattering (DLS). A metastatic model was established byinjecting Luc+ COLO 205 tumor cells into the tail vein of NOD/SCID mice. The mice receivedsystemic treatment with either TRAIL-LNP-Fuc or non-functionalized LNPs every two days.Tumorprogressionwasmonitoredviabioluminescenceimaging,flowcytometry,andconfocalmicroscopy. Tumor cell death was assessed by flow cytometry. **Results:** We developed afunctionalized LNP platform for targeted mRNA delivery to tumor cells. Functionalized LNPsshowed enhanced targeting to tumor cells both in vitro and in vivo. In the metastatic model,systemicadministrationoffunctionalizedLNPsinhibitedcoloncancerprogressionandmetastasis,reducingtumorburdenatlowdosages. TRAIL-LNP-Fucalsosignificantlydecreasedpulmonarymetastaticfociandimprovedtheanimals'weight.LNPfunctionalizationincreased active caspase-3 production in tumor cells, indicating programmed cell deathactivation. **Conclusion:** The functionalized LNP platform effectively delivers TRAIL mRNAspecifically to tumor cells, resulting in targeted tumor cell death. Clinically, this platform offerspotential for the targeted inhibition of tumor progression and metastasis, providing a newstrategyforsafeandeffectivesolidtumortreatment.

177. 466. **NOVEL STRATEGIES FOR HUMAN B-LYMPHOID NEOPLASMS USING SUPERANTIGENS (SAGS)**

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It has been reported (Lorenzo et al. 2016) that B-cell superantigens (SAgs) such as PpL induce apoptosis in malignant k+ B-cell lymphomas, in human cell lines, and murine models, demonstrating the potential use of superantigens as anti-cancer drugs, particularly when strategically paired with a specific Vk light chain. Our primary objective was to confirm these results in human cell lines of non-Hodgkin lymphomas (NHL) to determine whether these observations could be extrapolated to primary cultures of patients with leukemias and lymphomas diagnosed and well-characterized by flow cytometry at the Hospital de Clínicas José de San Martín, UBA. Once the effect of PpL was verified, we compared it with the effect of *S. aureus* enterotoxins G and I (SEG and SEI), two of the bacterial SAgs produced in our laboratory (Fernandez et al. 2006). The effect of SAgs was measured by thymidine incorporation and annexin V apoptosis assays. Specifically, SEG and, notably, SEI, affected the viability and/or proliferation of leukemias and lymphomas at 72 and 96H of treatment, similar to specific B-cell SAgs like PpL, with a significance of $p<0.0001$. Both SAgs were able to modulate the secretion of senescence-associated cytokines, such as IL-6, in the KM-H2 cell line, a Hodgkin lymphoma model ($p<0.0001$). Interestingly, for the more aggressive lymphoma or NHL, SAgs potentiated their effects when combined in vitro with other approved immunotherapies, such as humanized anti-CD20 monoclonal antibody or Obinutuzumab. As observed in lymphoma cell lines, PBMCs from patients with chronic lymphocytic leukemia (CLL) and NHL were shown to be more susceptible to treatment with SEI than with SEG. This finding could be explained by the fact that, unlike SEG, SEI possesses a β-chain zinc-binding site, which confers stronger binding to antigen presenting cells through MHC-II, as suggested by our preliminary interaction assays employing EDTA as a chelating agent.

178. 469. DEVELOPMENT OF A NANOBODY LIBRARY TARGETING SCORPION VENOM

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Envenomation incidents are a significant public health issue, particularly in tropical and subtropical regions. In Brazil, more than half of these incidents are caused by scorpions. Severe and moderate cases of scorpion envenomation require treatment with specific antivenom, which has been produced from horses since the early twentieth century. However, in the current context where animal health and welfare are essential, the need for a more efficient and less invasive production methods have driven the search for alternative technologies for the production of antivenoms and antitoxins. Llama-derived nanobodies (VHHs) have a great therapeutic potential for infectious, autoimmune and oncologic diseases. They are single-domain antibody fragments derived from camelids and have emerged as promising therapeutic agents due to their small size, stability, solubility in aqueous solutions, and high specificity and affinity for target antigens. These features, combined with their low toxicity and immunogenicity, make nanobodies a compelling alternative for antivenom production. The aim of this study was to develop a VHH-gene library from llama immunized with *Tityus serrulatus* scorpion venom. After the immunization, the llama developed a strong antibody response to the venom. Total RNA was extracted from the blood lymphocytes and a VHH-gene library of about 4.2×10^9 independent colonies and 100% of gene insertion was constructed. Nanobodies were then selected via phage display. Initial screenings have identified promising clones that are currently undergoing expression, purification and further testing of the potential to neutralize the toxic effects of the scorpion venom. The results of this study suggest that the immune nanobody library against *Tityus serrulatus* venom represents a valuable source of potential neutralizing antibodies against the venom. If successful, this approach could revolutionize the treatment of scorpion envenomations, offering a new generation of antitoxins that are both ethically sourced and economically viable.

179. 480. ARACHIS HYPOGAEA L. TEGUMENT ETHANOLIC EXTRACT MODULATES INNATE IMMUNE RESPONSE IN A MODEL OF INFECTION WITH DENGUE VIRUS

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In many infectious and non-infectious diseases, the immune response is deregulated. In viral infections such as dengue, the production of certain cytokines can be exacerbated, contributing to immunopathogenesis. *Arachis hypogaea* L. -peanut- has numerous medicinal properties. Therefore, peanut extract could regulate the host's immune response, improving its immune status and preventing diseases. The aim of this work was to evaluate cytotoxicity in human mononuclear cells and the immunomodulatory effect on pro-inflammatory and anti-inflammatory cytokines of the tegument ethanolic extract of peanut (TEE), in a model of infection with dengue virus serotype 2 (DENV2). The EET was obtained by simple alcoholic extraction method. For cytotoxicity and immunomodulation assays, peripheral blood mononuclear cells (PBMCs) from healthy human donors (Banco de Sangre, UNC) were used. Cells were obtained with Ficoll- Hypaque, seeded in culture plates with RPMI-1640 medium, and treated with TEE at concentrations from 50 to 200 µg/mL and incubated at 37°C for 48 h. Cell viability was determined by trypan blue exclusion. For immunomodulation, PBMCs were stimulated with DENV2 (50 PFU/mL) and treated with TEE at 10 and 20 µg/mL. Cellular (CC) and viral (VC) controls were included. After 48 h, supernatants were removed, centrifuged, and TNF-α, IL-6, and IL-10 production was assessed using ELISA kits. Statistical analysis was performed using GraphPadPrism 6.0. TEE cytotoxicity studies indicated a viability greater than 80% at concentrations ≤200µg/mL. Immunomodulation results revealed a significant increase ($p<0.05$) in cytokine levels (pg/mL) in VC in relation to CC for IL-6 (194±1.4vs164.5±6.3), TNF-α (526.9±23.9vs118.4±14.1) and IL-10 (1242.7±52.6vs142.3±9.7). TEE (20µg/mL) significantly decreased ($p<0.05$) the levels of cytokines with respect to VC: IL-6 (171.5±2.1 vs 194±1.4), TNF-α (353.8±3.9 vs 526.9±23.9) and IL-10 (766.9±23.9 vs 1242.7±52.6). In conclusion, *A. hypogaea* TEE modulate cytokines production at safe concentrations, which could have application in phytotherapeutic formulations or functional foods that improve the immune system.

180. 489. **IMMUNOTHERAPEUTIC ANALYSIS OF TWO PROTOCOLS AGAINST CANINE VISCERAL LEISHMANIASIS OVER 12 MONTHS**

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1. UNILA

Canine visceral leishmaniasis is a protozoan disease transmitted by sand flies and caused by the parasite Leishmania infantum. In Brazil, the country with the highest concentration of cases, there is only one drug licensed for this purpose in canines, miltefosine, which unfortunately already presents cases of parasitic resistance. Therefore, the objective of this study was to evaluate the ability to maintain the canine patient stabilized over 12 months using two immunotherapeutic protocols, with a formulation of total antigens of Leishmania amazonensis plus saponin (LaSap). In this study, a total of 48 dogs naturally infected with L. infantum were used, and they were categorized into patients with clinical stages grades 1 and 2, who received a protocol of four doses subcutaneously at 21-day intervals, and patients with clinical stages between grades 3 and 5, who received six doses, the first two at one-week intervals, the third dose 15 days after the second dose, and the last three doses at 30-day intervals between each dose. In both protocols, the control groups received only soluble antigen of L. amazonensis. All dogs were followed for 12 months after the end of the protocols, receiving a single booster dose every three months. For statistical analysis, the T test was used. As a result and a 95% confidence interval, it was verified that in both protocols there was a reduction in the parasite load in the skin and bone marrow, as well as greater production of IFN-gamma and less IL-10 in the supernatant of the PBMC culture. Clinically, patients in stages 1 and 2 maintained better compared to dogs in more advanced stages. In conclusion, these results indicate that immunotherapy with LaSap may be an alternative for stabilizing canine patients, without the risk of generating parasitic resistance.

181. 490. **MIFEPRISTONE ACTIVATES AN IMMUNOTHERAPY-RESPONSIVE PROFILE IN HORMONE-DEPENDENT LUMINAL BREAST TUMORS**

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During immunotherapeutic regimens in breast cancer, the endocrine context should be regarded as a critical contributor in the modulation of the immune function. It was shown that the sexual hormone progesterone (Pg) not only promotes breast cancer initiation, but also accentuates an immunosuppressive and tolerogenic environment. To counteract this suppression and modify the immune profile, we aimed to evaluate the capacity of Mifepristone (MFP), an antagonist of the progesterone receptor, to activate an immunotherapy- responsive profile in breast cancer. We assessed the immune landscape in a mouse model of luminal tumours (C4HD), as well as human tumour samples derived from the MIPRA clinical trial (NCT02651844), where breast cancer patients harboring Hormone Receptor positive tumours (PRA>PRB) were treated with MFP prior to surgery. We focused our research on the tumour immune infiltration profile, including bioinformatic analysis of various global gene expression signatures associated with T-cell exclusion and Immune Checkpoint Inhibitors (ICI) resistance by means of RNA-seq and flow cytometry. We noticed that MFP treatment in C4HD tumors mitigates the progestin-mediated tolerogenic infiltrate, which includes regulatory T cells, exhausted CD8+ T cells and M2 macrophages. Notably, MFP downregulates the suppressive pathway of IDO and Galectin-9 in tumours, thereby contributing to the persistence of a CD8+ T population that exhibits a reinvigorating phenotype characterized by high granzyme production and low expression of TIM3 and PD-1. Specifically, our findings demonstrate that in both mouse models and human tumours, MFP treatment reverses transcriptional programs associated with T cell exclusion and ICI resistance while significantly upregulating programs associated with PD- 1/ PD-L1 response. Our work provides a foundation for a novel therapeutic approach based on the use of anti-progestin endocrine therapy with MFP toward sensitizing luminal breast tumours to ICI treatment.

182.497. AFFINITY MATURATION OF ANTI-MICA AND ANTI-SST2 ANTIBODIES THROUGH IN SILICO AND EXPERIMENTAL METHODS

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Background: Affinity maturation represents a crucial stage in the development of antibodies, with the objective of enhancing their binding affinity. While traditional methods of affinity maturation are effective, they are often limited by time-consuming and resource-intensive experimental processes. To address these limitations, *in si/ico* affinity maturation methods have emerged as a means of accelerating and enhancing the antibody development process. **Objective:** In this study, we employed an in silico affinity maturation process with the objective of enhancing the binding affinity of an anti-MICA and an anti-sST2 antibody far their target epitope. **Methods:** Computational methods were employed to model and optimize the structure of both antibodies and their interaction with their antigen. The Fvs were modeled using RosettaAntibody. The complexes were docked using ClusP ro2.0 and SnugDock. Furthermore, molecular dynamics
simulations were conducted to evaluate the Gb ni d ni g using MMGBSA. Subsequently, all the antibodies were expressed in ExpiCHO cells. Finally, the binding affinity of the was assessed by ELISA. **Results:** We identified 30 and 10 mutation sites within the CDRs of the anti-MICA and the anti-sST2 antibody, respectively. These sites were subjected to mutations by 10 amino acids, resulting in 300 and 100 mutants far the anti-MICA and the anti-sST2 antibody, respectively. Five mutants of each antibody that showed enhanced *in si/ico* binding affinities were expressed in CHO-S cells in arder to validate their experimental bind-

ing affinity. **The Elisa assays demonstrated that the mutant 129DL [1] of the anti-MICA antibody and mutants ES0 HH [2] S57FL [2] and Y33AH [1] of the anti-sST2 antibody showed an enhanced affinity up to 1000 times compared to the wild type. Conclusions:** Mutants with enhanced affinity were obtained far both antibodies, thereby demonstrating that this integrative approach provides a comprehensive understanding of the antibody-antigen interaction and establishes a solid basis far the rational design of anti bodies.

183. 502. MODULATORY ROLE OF SMALL EXTRACELLULAR VESICLES-OBTAINED FROM INDUCED-NEUROPILIN-1+ T REGULATORY CELLS

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Background: T regulatory cells (Tregs) are a subset of CD4+ T cells that modulate the immune response to maintain self-tolerance and immune homeostasis. Naïve CD4+ T cells can be induced *in vitro* to Tregs (iTregs). Tregs can suppress immune responses through the release of small extracellular vesicles (sEV). Previously, our group described that sEV from nTregs lacking Neuropilin-1 (*Nrp1KO*) have an altered suppressor function *in vitro* and *in vivo.* Objective: We evaluated the production of sEV from iTregs and the contribution of Nrp1 on CD4+ T cells function (conv T cells). Methods: Wild Type (*WT*) and *Nrp1KO* naïve T cells were purified using magnetic beads and cultured for 5 days with IL-2+TGFβ (iTregs). Supernatants were ultracentrifugated and sEV were purified using IZON columns. Nanoparticle tracking analysis was used to analyze size and number of particles. *In vitro* modulation assay was performed on polyclonally activated CTV-conv T cells in the presence of sEV from *WT* and *Nrp1KO* iTregs. Cell proliferation and phenotype were studied by flow cytometry, and production of IFN-y, IL-17 and IL-10 were measured by ELISA. Results: iTregs produced from *Nrp1KO* CD4+ T cells gained less expression of FoxP3 than *WT* CD4+ T cells. sEV from *WT* iTregs suppress conv T cell proliferation and CD25 expression, and induce FoxP3, Nrp1, CD44 and ST2, although no statistical significance was obtained. sEV from *Nrp1KO* iTregs are less effective inducing FoxP3 and Nrp1, and in blocking CD25 expression on conv T cells. Last, sEV from *WT* iTregs down-regulate IFN-y only, whereas sEV from *Nrp1KO* iTregs tend to induce IL-17 and block IL-10, with no effect on IFN-y production. Conclusions: Nrp1 is required for optimal induction of iTregs. On sEV, Nrp1 is necessary for stimulating a regulatory phenotype on conv T cells given by the expression of FoxP3, Nrp1, CD25, IL-17 and IL-10.

184. 516. TOTAL TUMOR MRNA TRANSFECTION INTO MO-DCS FOR BREAST CANCER THERAPY

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Background: Breast cancer still ranks in second among causes for cancer related death in women. Patients with metastatic, aggressive and/or relapsed disease remain a challenge to clinicians, with new therapeutic approaches being needed. Immunotherapy has been considered as an interesting strategy, more specifically, the development of dendritic cells-based vaccines. **Objectives:** The present work focuses on standardize total tumor mRNA expansion *in vitro* and test three different types of lipoplexes for its transfection into mo-DC, as a first step of an immunotherapy strategy for breast cancer. **Methods:** SK-BR3 cells were cultured and had their RNA extracted with TRIzol for cDNA synthesis and amplification *in vitro*. After, mRNA transcription was performed with a CAP 5' addition. Mo-DCs were differentiated from PBMCs of healthy donors and transfected with 5 µg of RNA using three types of lipoplexes – Lipofectamine 2000™, 3000™ and FUGENE®, at two different volumes each (10 and 15 µl). Flow cytometry was performed after 24 hours for Her2/neu protein, as an indicator of the transfection procedure. Non-transfected mo-DCs and mo-DCs transfected just with each vehicle were kept as controls **Results:** Our preliminary results indicate that total tumor mRNA could be amplified *in vitro*, with a final concentration of 899,9 ng/µl and a A260/280 of 1,68. Flow cytometry data indicates that Lipofectamine 2000™ presented the lowest toxicity – 54,2% of live cells after transfection, compared with 50,4% from Lipofectamine 3000™ and 44,5% from FUGENE®. There were no differences in Her2/neu expression between controls and transfected cells in any of the conditions tested. **Conclusions:** total tumor mRNA can be amplified *in vitro*, although purifi-

cation may be needed for the removal of remaining cDNA. The lack of Her2/neu expression on transfected mo- DCs after 24 hours may indicate that there was no sufficient time for the protein to be translated and expressed on the membrane. Our next set of experiments will include 40- and 48-hours post- transfection analysis.

185. 517. CHARACTERIZING THE BINDING AFFINITY AND SPECIFICITY OF A FULLY HUMAN ANTI-MICA MONOCLONAL ANTIBODY

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MHC class I polypeptide-related sequence A (MICA) is a highly polymorphic protein with numerous allelic variants. It is overexpressed on the membrane of a variety of tumor cells. MICA is a key ligand for the NKG2D activating receptor on natural killer cells, leading to tumor cell lysis. However, as an immune evasion mechanism, MICA can be released into the extracellular medium, where it interacts with the NKG2D receptor and induces its down-regulation, thereby preventing tumor cell recognition. Consequently, the neutralization of soluble MICA has been proposed as a potential therapeutic target. The objective of this study was to evaluate the binding capacity of a fully human anti-MICA antibody to allelic variants of MICA expressed on gastric adenocarcinoma cells, as well as its ability to block MICA and NKG2D receptor interaction. The capacity of the antibody to bind MICA and block the MICA-NKG2D interaction was evaluated using ELISA. Furthermore, gastric cell lines GES-1, MKN-45, and AGS, were used to assess the binding capacity of the anti-MICA antibody in comparison to a commercial murine anti-MICA antibody, by flow cytometry. We demonstrated that our anti-MICA antibody binds to the soluble form of MICA and competes for MICA binding with the NKG2D receptor. Furthermore, while both antibodies exhibited comparable levels of MICA detection in GES-1 cells (MICA*008), the levels of MICA detected by our anti-MICA antibody in MKN-45 (MICA*009) and AGS (MICA*010) cells were found to be higher than those detected by the commercial anti-MICA monoclonal antibody

In conclusion, the anti-MICA antibody demonstrated recognition of more allelic variants of MICA. The binding capacity of the anti-MICA antibody to the majority of MICA alleles and its ability to compete with NKG2D could serve as a potential platform for further development of a therapeutic antibody in oncology.

186. 518. EVALUATION OF EFFECTOR MECHANISMS OF A NOVEL FULLY HUMAN ANTIBODY TARGETING THE MICA PROTEIN IN GASTRIC CANCER CELL LINES

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Background: The use of monoclonal antibodies for cancer treatment has become one of the main immunotherapeutic strategies, due to their high affinity and antigenic specificity, as well as their ability to interact with receptors on various immune system cells via the crystallizable fragment (Fc). The mechanisms activated include complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent cellular cytotoxicity (ADCC), all of which are involved in the selective elimination of tumor cells. The design of monoclonal antibodies has focused on different targets, including MICA, a transmembrane protein that is overexpressed in a wide range of tumor types, representing a promising target for cancer therapeutic intervention. Our approach involves the use of a rationally designed, fully human anti-MICA IgG1 antibody, which is expected to induce

selective killing of tumor cells via Fc effector functions (ADCP and CDC). **Methods:** In this study, EGFP-expressing U937 monocytic cells were differentiated into macrophages by the addition of phorbol 12-myristate 13-acetate (PMA). Gastric cell lines MKN-45, GES1, and AGS, known to express MICA, were labeled with a fluorescent probe, incubated with our anti-MICA antibody, PBS (vehicle), or rituximab (isotype control), and subsequently co-incubated with macrophages. Macrophage phagocytic efficacy was evaluated using flow cytometry and confocal microscopy. CDC was evaluated in MKN gastric cells, which were incubated with the anti-MICA antibody and serum samples from healthy donors. Cell viability was then assessed by flow cytometry using a viability probe. **Results:** Our results demonstrate an enhancement in macrophage-mediated phagocytosis of gastric cancer cells with the anti-MICA antibody compared to vehicle and isotype controls. Furthermore, a decrease in cell viability was observed in groups treated with the anti-MICA antibody. **Conclusion:** These results suggest that the anti-MICA antibody enhances immune effector mechanisms against MICA-expressing tumor cells, validating the biotechnological potential of the anti-MICA antibody as a potent anti-tumor biopharmaceutical.

187. 562. **MOLECULAR CHARACTERIZATION OF SINGLE-CHAIN VARIABLE FRAGMENT ANTIBODIES WITH THERAPEUTIC POTENTIAL TARGETING ALPHA-2 DOMAIN OF MICA**

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MICA is a highly polymorphic protein, and its soluble form has been identified as a potential target in oncology due to its ability to facilitate immune evasion by reducing the expression of the NKG2D receptor on natural killer cells. In this context, two single-chain variable fragments (scFv) targeting the alpha-2 domain of MICA, named clone-1 and clone-7, were selected from a phage display library with the aim of neutralizing the binding of the NKG2D receptor to MICA. However, a more in-depth characterization of the molecular binding mechanism of both antibodies is required to select the one with the desired therapeutic potential. The aim of the study was the structural characterization of two scFvs antibodies through *in silico* and *in vitro* experimentation Anti-MICA scFvs and MICA were expressed in *Escherichia coli* BL21(DE3) cells, purified from inclusion bodies and then refolded *in vitro*. The affinity of the antibodies and their ability to displace NKG2D binding by MICA were determined by ELISA. The binding to MICA alleles *002, *008, and *009 were verified by flow cytometry. The structure of the Fv fragments was modeled using RosettaAntibody, and the structure of the Fv-MICA complex was predicted through molecular docking using ClusPro and SnugDock It was determined that the affinity constants of the antibodies clone-1 and clone-7 are 67 and 80 nM, respectively. However, only the scFv clone-1 has the ability to displace the binding of NKG2D to MICA but it was unable to recognize allele *009. The *in silico* model predicts that only clone-1 has an epitope on MICA that overlaps with NKG2D while clone-7 binds to a distant epitope. The combined analysis of *in vitro* and *in silico* experimentation allowed us to select a high-affinity anti-MICA antibody with therapeutic potential and characterize its molecular mechanism.

188. 569. **CHIMERIC ANTIGEN RECEPTOR (CAR) ENHANCED FUNGICIDE ACTIVITY OF MACROPHAGES AGAINST CRYPTOCOCCUS SP.**

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Background: Cryptococcosis is a systemic fungal infection caused by *Cryptococcus neoformans* or *Cryptococcus gattii*; immunocompromised patient and healthy individuals are affected, leading to severe pulmonary infections and/or meningoencephalitis. Conventional therapy involves antifungal drugs that exhibit high side effects and antifungal drugs resistance is a concern, then alternative immunotherapeutic approaches is required. Immune cells engineered with Chimeric Antigen Receptors (CAR) has emerged as a promising cell therapy approach against fungal infections, and the generation of CAR-Macrophages against *Cryptococcus* sp. is a pioneering study to improve the control of cryptococcosis. **Objectives:** Evaluate macrophages expressing CAR, which is specific to *Cryptococcus* sp., containing Dectin-1 (GXMR-Dectin-1-CAR) or FcγRI (GXMR-FcγRI-CAR) as signaling domain to trigger pathways required for effector antifungal immunity mediated by macrophages against cryptococcosis. **Methods:** Alveolar macrophages AMJ2-C11 and MH-S were transduced with GXMR- FcγRI-CAR or GXMR-Dectin-1-CAR, and these cells were co-cultured with *Cryptococcus* sp. (H99 or R265 strains) before the determination of the activation profile, and phagocytic and microbicide activity of CAR-macrophages. **Results:** Cells modified with GXMR-FcγRI and GXMR-Dectin-1 exhibited high levels of TNF-α and IL-6 production upon incubation with *Cryptococcus* sp., and Syk inhibitor evidenced the involvement of Syk pathway in the CAR-macrophages activation. GXMR-FcγRI increased the *Cryptococcus* sp. phagocytosis and improved the adherence to Titan form of *Cryptococcus* sp. GXMR-FcγRI-CAR and GXMR-Dectin-1-CAR mediated a reduction in the intracellular proliferation rate of *Cryptococcus* yeasts, and this fungicide activity can be associated with the macrophage subsets as demonstrated by polarization markers measured by qRT-PCR and flow cytometry. **Conclusion:** Alveolar macrophages AMJ2-C11 and MH-S expressing GXMR-FcγRI-CAR or GXMR-Dectin-1-CAR target yeast and titan forms of *Cryptococcus* sp., and GXMR-FcγRI-CAR improved the phagocytic and fungicide activity against *Cryptococcus* sp. opening a new avenue in the CAR-cell therapy application to treat cryptococcosis.

189. 580. GENERATION AND VALIDATION OF AN ANTI-SST2 SCFV: DEVELOPMENT OF A THERAPEUTIC ANTIBODY FOR ULCERATIVE COLITIS

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Introduction: Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by an exaggerated immune response, leading to cycles of activity and remission. Single-chain variable fragments (ScFv) and monoclonal antibodies have emerged as promising therapeutic strategies. The sST2 protein, integral to the IL-33 signaling pathway, plays a critical role in UC's pathophysiology. Blocking sST2 is crucial because it acts as a decoy receptor, binding IL-33 and preventing it from triggering anti-inflammatory responses, thereby exacerbating inflammation. Targeting sST2 could potentially restore IL-33's beneficial effects, reducing inflammation and improving patient outcomes. **Objectives:** This study aims to generate and validate an ScFv

targeting sST2 (ScFvα-ST2) and to develop a fully human monoclonal antibody (AcHu-αsST2) derived from ScFvα-ST2. The therapeutic potential of AcHu-αsST2 will be evaluated by assessing its ability to modulate cytokine levels in UC patient biopsy samples. **Methodology:** ScFvα-ST2 was generated using bacterial expression systems, purified, and its binding affinity to sST2 was validated using immunoprecipitation and ELISA. Subsequently, ScFvα-ST2 was used to develop the monoclonal antibody AcHu-αsST2, produced in CHO-K1 cells. The therapeutic potential of AcHu-αsST2 was assessed by treating UC patient biopsy samples and measuring changes in cytokine levels, particularly IL-10 and TNFα. **Results:** ScFvα-ST2 was successfully purified to 97% purity and demonstrated strong binding affinity to sST2 in HMC1 cells, with an 88.9% detection rate. AcHu-αsST2 significantly increased IL-10 levels and decreased TNFα levels in UC patient biopsies, indicating reduced inflammation. **Conclusion:** The characterization and validation of ScFvα-ST2 and the therapeutic evaluation of AcHu-αsST2 suggest that targeting the IL-33/ST2 pathway could offer a promising treatment strategy for UC by modulating immune responses.

190. 591. **HIGHLY PURIFIED AND FUNCTIONALLY STABLE ALLOANTIGEN-SPECIFIC FOXP3+CD27+CD70- REGULATORY T CELLS WITH POTENTIAL IMMUNOTHERAPEUTIC APPLICATION IN SOLID ORGAN TRANSPLANTATION.**

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Preclinical studies show that alloantigen-specific FOXP3+ regulatory T cells (AE-Tregs) are more effective at inducing allograft tolerance compared to other treatments, including immunosuppressive drugs that, in the long term, cause adverse effects in transplanted recipients. The clinical application of AE-Tregs in solid organ transplantation has been limited due to their low frequency in peripheral blood and the scarcity of available protocols that allow to obtain enough AE-Tregs, ensuring their high purity and functionality after *ex vivo* expansion. To increase the yield, purity, and stability of AE-Tregs, The objective of this work was to isolate and expand AE-Tregs, producing Tregs with significant immunoregulatory capacity. To achieve this, we first co-cultured Tregs with allogeneic dendritic cells in the presence of IL-15 and IL-2. Then, proliferating CD27+CD70- AE-Tregs were FACS-sorted and polyclonally stimulated with anti-CD3/CD28 in the presence of IL-15, IL-2, rapamycin, and TGF-β. After three weeks, AE-Tregs expanded up to 500 times their initial numbers with a purity of >95% (FOXP3+) and high expression of immunoregulatory molecules such as CD27, CD25, CTLA-4, Helios, and CD39, which was related to their efficient ability to suppress T-cell proliferation in an antigen-specific manner. Importantly, AE-Tregs showed high expression of chemokine receptors relevant for allograft homing (CCR4 and CXCR3), as well as for migration to lymph nodes (CCR2 and CCR7). Finally, AE-Tregs maintained their immunosuppressive phenotype and function after being stimulated for an additional week with inflammatory cytokines (IFN-γ, IL-1β, IL-6, or TNF-α). In conclusion, we demonstrated the feasibility of obtaining a cellular product for customized Treg therapy in transplanted patients. This product is currently being scaled up to comply with GMP standards and will be evaluated in Phase 1 clinical trials.

191. 610. **THE ROLE OF TGF-B-INDUCED TIFL-Y AND SMAD4 ON REGULATORY T LYMPHOCYTES WITHIN THE TUMOR MICROENVIRONMENT.**

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TGF-B induces the proliferation and differentiation of regulatory T lymphocytes within the tumor microenvironment, resulting in the inhibition of effector lymphocytes. TGF-B signaling is mediated by a protein complex called Smad2/3/4 that translocates to the nucleus and induces gene ex-

pression. The transcription factor TIFI-y can interact with the Smad2/3 complex changing the outcome of gene induction and resulting in a different transcription profile and phenotype. Here, we aim to characterize the role of TIFI-y and Smad4 in TGF-B-induced tumor-specific regulatory T cells. We generated a transgenic mouse strain with antigen-specific regulatory T lymphocytes. In a model of melanoma, we found that tumor-infiltrating regulatory T lymphocytes express less TIFI-y than SMAD4. We then isolated the regulatory T lymphocytes and deleted SMAD4 and TIFI-y with CRISPR/Cas9. Functionality assays of edited lymphocytes showed that Smad4-deficient cells maintain their stability and suppressive capacity compared to TIFI-y-deficient cells. The SMAD4- or TIFI-y-deficient regulatory T lymphocytes were adoptively transferred into mice with melanoma tumors with a positive impact on tumor growth. These results position SMAD4 and TIFI-y as therapeutic targets against cancer.

192. 627. EFFECT OF MESENCHYMAL STEM CELLS-DERIVED EXTRACELLULAR VESICLES ON B CELLS

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Background: Autoimmune diseases (ADs) are characterized by B cell function dysregulation. Novel therapies such as antibody-mediated B cell depletion have significant side effects. Mesenchymal stem cells (MSCs) have been proposed as an alternative therapy for ADs, as these cells bear potent immunomodulatory capacities, particularly when exposed to inflammatory conditions known as 'licensing'. MSCs-derived extracellular vesicles (EVs) have been proposed as safer alternative to MSCs. **Objective**: The aim of this study was to evaluate the effect of EVs derived from licensed and unlicensed MSCs on B cell function and activation **Methods:** Bone marrow murine MSCs (BM-MSCs) were cultured on tissue culture plastic in EV-depleted media with IFN-γ and TNF-α to 'license' them, or in standard conditions for 48 hours. The conditioned media was then collected, and the MSCs-EVs were isolated by size exclusion chromatography. EVs samples' particle concentrations and protein concentrations were measured by NTA and BCA, respectively. For assessing the effect of EVs on B cells, primary B cells were isolated from an allogeneic strain mouse and activated with CD40L, or CpG. The effect of licensed and unlicensed EVs on the expression of activation surface markers and proliferation of B cells was measured by spectral flow cytometry. **Results:** 'Licensed' and 'unlicensed' BM-MSCs EVs could modulate the expression activation markers MHCII, CD80, CD69 and CD86 on allogeneic murine B cells activated both by CD40L and CpG. EVs were also capable of modulating B cell proliferation. **Conclusion:** Our findings demonstrate that BM-MSCs-derived EVs can modulate B cell activation and function *in vitro*. Moreover, our study highlights the influence of MSCs' culture conditions on EV effects, suggesting potential therapeutic versatility in diseases characterized by immune dysregulation, such as ADs. Further elucidation of the underlying mechanisms is crucial to fully harness the clinical potential of MSCs-derived EVs as immune therapies.

193. 650. FEATURING A NOVEL NATURAL IMMUNOSTIMULANT: THE HEMOCYANIN OF POMACEA CANALICULATA

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Hemocyanins are colossal proteins that act as respiratory pigments in invertebrate physiology. Particularly, mollusk hemocyanins have raised interest for their immunostimulant properties over mammals, a trait that has been related to their enormous size and unusual glycosylation that resembles tumor associated antigens. Nevertheless, besides the widely known keyhole limpet hemocyanin (KLH), successfully employed as an adjuvant in vaccine formulations and non-specific immunostimulant for bladder cancer therapies, few other hemocyanins have been biochemically and immunologically characterized. Since hemocyanins are obtained from natural sources and no recombinant forms currently exist, bioavailability

poses a challenge to face their increasing demand. In this context, we aimed to investigate the immunogenic properties of the hemocyanin of the snail *Pomacea canaliculata* (PcH), a freshwater South American species. Employing flow cytometry, fluorescence microscopy, immunoassays, and quantitative PCR, we analyzed PcH effect on THP-1 monocytes and their derived macrophages, as well as its capability to induce humoral response on C57BL/6 mice. Additionally, we inquired PcH structural stability against temperature and pH variations by spectroscopy analysis. As a result, we found that PcH is a structurally stable protein that triggers a pro-inflammatory effect on THP-1 derived-macrophages by increasing IL1-β and TNF-α levels. Of interest, protein deglycosilation retrieved a relative decrease in cytokine secretion. Moreover, PcH was able to promote monocyte-to-macrophage differentiation, as evidenced by the morphological and immunometabolic changes observed in treated cells and the expression pattern depicted. Finally, the PcH-induced humoral response was indistinguishable from that of KLH, highlighting its promising features. Taken together, our results point out PcH as a promising alternative in the search for new immunostimulant compounds, especially considering it derives from a freshwater species, simple to rear and maintain, with an easy-to-scale purification method.

194. 690. **STUDY OF THE IMMUNOSUPPRESSIVE POTENTIAL OF EXTRACELLULAR VESICLES SECRETED BY METABOLICALLY REPROGRAMMED MESENCHYMAL STEM CELLS WITH A GLYCOLYTIC PROFILE ON B LYMPHOCYTES IN VITRO**

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Mesenchymal Stem/Stromal Cells (MSCs) are multipotent stromal cells capable of differentiating into specialized cell types, contributing to tissue repair. MSCs also possess significant immunomodulatory and immunosuppressive properties, achieved through both direct cell-to-cell contact and their secretome, including extracellular vesicles (EVs). These EVs are crucial for immune regulation, transferring immunosuppressive molecules from MSCs to target cells like macrophages and T lymphocytes. However, their impact on B lymphocytes is less understood. Recent studies demonstrated that reprogramming MSCs to a glycolytic metabolism enhances their immunosuppressive activity, particularly towards T lymphocytes. However, the effects of glycolytically reprogrammed MSC-EVs on B lymphocytes remain unexplored. Objetive: To assess the immunosuppressive capacity and viability of extracellular vesicles secreted by Mesenchymal Stem Cells reprogrammed to a glycolytic metabolism on activated B lymphocytes *in vitro*. MSCs were isolated from the umbilical cord of healthy donors and treated with oligomycin for 24 hours to induce a glycolytic metabolism (MSCgly). EVs were isolated from MSCgly through ultracentrifugation, quantified using nanoparticle tracking analysis, and characterized by transmission electron microscopy and flow cytometry. B lymphocytes were isolated from peripheral blood of healthy donors, sorted and culture *in vitro*. EVs were added to the culture medium of activated B lymphocytes, with viability assessed using annexin V and PI assays at 24, 48, and 72 hours. Additionally, activation, proliferation, and differentiation of B lymphocytes were evaluated 72 hours after activation and treatment with MSCgly-EVs using flow cytometry. Metabolic reprogramming does not affect the characteristics of MSC-EVs. We observed increased viability of B lymphocytes treated with EVs, particularly those derived from glycolytic MSCs. This suggests that EVs from glycolytically reprogrammed MSCs enhance B lymphocyte viability and may lead to suppression of their function. These findings underscore the potential of MSCgly-EVs in developing new therapeutic strategies for autoimmune diseases involving hyperactive and proliferative B lymphocytes.

195. 708. **PSGL-1 EXPRESSING MYELOID CELLS INHIBIT MELANOMA ANTI-TUMOR IMMUNITY**

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P-selectin glycoprotein ligand-1 (PSGL-1) has emerged as a pivotal regulator of immune responses, particularly in the context of cancer immunotherapy. Despite the widespread use of immune checkpoint inhibitors, a significant subset of patients remains unresponsive to these treatments, highlighting the need for alternative targets. This study investigates the therapeutic potential of PSGL-1 and VISTA, another immune

checkpoint molecule, in a PD-1-therapy resistant melanoma model. We demonstrate that both anti- PSGL-1 and anti-VISTA therapies effectively inhibit tumor growth, although no synergistic effects were observed. Interestingly, conditional deletion of PSGL-1 in myeloid cells, including macrophages and dendritic cells, revealed its critical role in modulating the tumor microenvironment. The loss of PSGL-1 in myeloid cells led to enhanced T cell responses and reduced immune suppression, which delayed melanoma tumor growth. These findings suggest that PSGL-1 inhibition can reprogram the immune landscape, offering a novel strategy to overcome resistance to existing therapies. The dual role of PSGL-1 in regulating both T cell function and myeloid cell activity underscores its potential as a highly impactful immunotherapeutic target in cancer treatment.

INFECTION AND IMMUNITY

196. 009. THE IMPACT OF HYBRID IMMUNITY ON MEMORY T CELL RESPONSES AGAINST SARS-COV-2 IN A COHORT OF VOLUNTEERS IN MEXICO CITY

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Introduction: The COVID-19 pandemic has deepened our understanding of protective mechanisms against SARS-CoV-2, emphasizing both T cell- and antibody-mediated immunity. In Mexico, vaccination efforts have notably reduced hospitalizations and fatalities. However, concerns about immunity duration and new variants have prompted booster vaccination campaigns. Notably, an enhanced immune response has been observed in individuals who recovered from COVID-19 and received vaccination, or in vaccinated individuals who later contracted the infection—termed "hybrid immunity"—with increased neutralizing antibody titers. This raises questions about potential impacts on T cell memory profiles, effector capacity, and longevity. **Objective:** This study aims to characterize the memory T cell response induced by hybrid immunity against SARS-CoV-2 in a cohort of volunteers in Mexico City. **Methods:** Cellular and IgG antibody responses were assessed in 80 volunteers throughout the SARS-CoV-2 vaccination program. Flow cytometry was employed to evaluate memory subpopulations, while ELISA was utilized to quantify the production of specific antibodies targeting viral proteins. **Results:** Similarities were observed in the memory subpopulations of vaccinated individuals and those with hybrid immunity, characterized by a predominance of CD4+ effector memory T cells 3 (TEM3) and CD8+ TEM2 cells. Additionally, volunteers with hybrid immunity exhibited increased IFN-γ production in TCM and TEMRA, along with a slight rise in IL-2 production in TEM2 and TEM3 memory cells. Moreover, an augmentation in CD8+ T cell populations was noted, indicating an enhanced effector response attributed to hybrid immunity development. Lastly, individuals with hybrid immunity displayed elevated IgG antibody titers. **Conclusion:** In a cohort of volunteers in Mexico City, hybrid immunity boosts the effector capacity of CD8+ T cells, potentially playing a crucial role in the immune response against SARS-CoV-2 and the effectiveness of booster vaccination campaigns, even with first- generation vaccines.

197. 014. POST SEPSIS EXERCISE THERAPY ENHANCES CD4 T CELL RECOVERY IN THE LUNG.

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Sepsis survivors often develop persistent impairment in immune functions, known as immunosuppression, thus elevating the risk of recurrent infections. Currently, there is no effective approach to reverse this immunosuppression and prevent recurrent infections in sepsis survivors. While exercise therapy has demonstrated positive effects on post-sepsis recovery of muscular and cardiorespiratory functions, its impact on immunosuppression remains unclear. C57BL/6 mice were subjected to cecal ligation and puncture (CLP) with antibiotic bid for 3 days to induce sepsis. After a one-week recovery, survivors

were subjected to post-sepsis exercise therapy (PSET) or sedentary (Sed) training for 4 weeks, followed by pseudomonas aeruginosa (PA, $5X10^7$ CFU in 50ul PBS, i.n.). The survival rate of the CLP model is 60%. We found that the percent of CD4 T cells persistently decreased from 24h to 5wk after CLP and returned to the control level 10 weeks after CLP. Furthermore, the percent of IFNY+ CD4 T cells was lower in CLP mice than in control mice. These data suggest that our model mimics the key feature of immunosuppression. Of note, PSET protected sepsis-surviving mice against secondary PA infection by reducing bacterial load in the lung and IL-6 and protein levels in the bronchoalveolar lavage fluid 24 hours after infection. Furthermore, PSET increased the percent of CD4 T cells in the lung compared with the Sed group. Using single cell RNA-sequencing, we found that CD4 T cells in Sed-mice upregulated gene expression enriched in ribosome biosynthesis pathway compared with the naïve control. PSET drove the transcriptomic profile of CD4 T cells towards the naïve control. Compared with the Sed group, PSET upregulated the expression of genes relevant to CD4 T cell activation and proliferation. Our results indicate that four-week PSET protected against secondary PA infection via enhancing CD4 T cell recovery in the lung. Understanding the mechanisms underlying the immunomodulatory effects of PSET will help us to design novel exercise-mimicking strategies for exercise-intolerant patients to prevent and treat recurrent infections.

198. 017. **INCREASED ESAT-6-SPECIFIC PHAGOCYTIC ACTIVITY AND DECREASED FC GAMMA RECEPTORS EXPRESSION AND IGG4 LEVELS IN CLINICALLY CURED TB PATIENTS**

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Tuberculosis (TB) is a leading cause of death and morbidity worldwide despite the availability of treatment. The role of humoral immune response in TB infection is still considered controversial. Few studies have explored antibody-mediated functions in TB treatment. Here, we focused on evaluating the impact of anti-TB treatment on antibody features and Fc gamma receptor expression on immune cells through serology and flow cytometry approaches. We follow up with active pulmonary TB patients who received standard treatment at three time points: before initiating treatment (T0), two months after starting treatment (T2), and at the end of treatment, after six months (T6). For the first time, we described that ESAT-6-specific antibodies show an enhanced ability to mediate phagocytosis in cured TB patients. Additionally, decreased levels of ESAT-6-specific IgG4 and activating receptors CD16, CD32, and CD64 on monocytes $CD14^+$ and CD64 on granulocytes $CD66b^+$ were observed upon successful treatment. These results underscore the potential of Fc receptor expression on monocytes, granulocytes, ESAT-6 specific IgG4, and phagocytic activity as potential biomarkers for follow-up patients undergoing treatment. Also, we provide critical insights about the antibody response on active pulmonary TB, and implications at the end of treatment should be further investigated.

199. 022. **THE CO-ADMINISTRATION OF AZITHROMYCIN AND EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STEM CELLS IMPROVES CLINICAL AND HISTOPATHOLOGICAL SCORES OF SEPTIC MICE**

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Background: Sepsis is a syndrome that occurs following an infection and marked by severe inflammatory responses, and if not treated in time, it can lead to multi-organ failure syndrome and death. Azithromycin (AZT), besides its antimicrobial properties, has anti-inflammatory effects and mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) show immunomodulatory effects. This study examines the impact of combining azithromycin and MSC-EVs on sepsis in a mouse model. **Objectives:** We decided to investigate the effects of a combinational therapy consists azithromycin and MSC-EVs in the mouse model of sepsis. **Methods:** Human Wharton's jelly-mesenchymal stem cells were cultured, characterized, and used to extract MSC-EVs. A cecal ligation and puncture (CLP) sepsis mod-

el was induced in mice, followed by treatments: saline, AZT, MSC-EVs, and combination therapy (AZT+MSC-EVs). Clinical sepsis scores were recorded 24 hours post-treatment. Serum, peritoneal fluid, and organ tissues (kidney, liver, lung) were analyzed for biochemical parameters, inflammatory markers, bacterial load, and histopathological changes. **Results:** The combinational therapy group showed significant improvements in clinical symptoms, reduced bacterial load, and decreased neutrophil-to-lymphocyte ratio (NLR) and TNF-α serum levels compared to the CLP group. Histopathological assessments revealed that the combination therapy significantly alleviated tissue damage in the liver, kidneys, and lungs. Biochemical analysis indicated a substantial decrease in AST and creatinine levels, highlighting improved liver and kidney functions. **Conclusion:** The combined use of azithromycin and MSC-EVs offers a promising therapeutic approach for sepsis by effectively controlling infection and modulating the inflammatory response.

200. 029. **PROGRAMMING CHECKPOINT BLOCKADE EFFICACY THROUGH THE DENDRITIC CELL-CYTOKINE AXIS**

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Background: Stem-like progenitor exhausted CD8 T cells (TPEx) are critical for maintaining long-term resistance during chronic infections and cancer, and represent an important checkpoint blockade immunotherapy target for functional reinvigoration and disease control. Hence, there is vigorous interest in understanding their ontogenesis. Objectives: To elucidate the cytokine signals that promote the development of long-lived checkpoint blockade responsive TPEx cells. Methods: We engaged a combination of chronic viral infection and solid tumor immunotherapy systems to characterize T cell fates and therapy responsiveness under differential cytokine signaling conditions. Results and Conclusions: Here, we show a crucial fate-deterministic role of rheostatic IL-2 signals and differential DC priming in programming the development of stem-like progenitor exhausted CD8 T cells during chronic viral infection. In vivo fate-tracking studies reveal that strong IL-2 signals during priming drive terminal differentiation. In contrast, tempered IL-2 signals are associated with TCF-1Hi stem-like precursors, which give rise to TPEx cells, capable of long-term persistence and potent responsiveness to anti-PD-1 therapy in later stages of chronic viral infection. In the context of tumors as well, scRNA-seq analyses of total or antigen-specific tumor infiltrating lymphocytes from melanoma, HPV+ head and neck cancer, and lung cancer patients show an inverse relationship of IL-2 signaling signature with T cell stemness, as well as checkpoint blockade therapy outcomes in melanoma. Our studies further show that the rheostatic control of exhausted T cell fates by differential IL-2 signals is physiologically mediated through differential cell surface expression of IL-2Ra during early stages of T cell activation, which in turn is pioneered during priming by distinct DC subsets. Notably, moderation of *in vivo* IL-2 signals during priming promotes the development of stem-like TCF-1Hi lineage, thus supporting a unique strategy for improving clinical immunotherapy outcomes by enhancing the development of long-lived, therapy-responsive cells with vigorous effector expansion capabilities.

201. 046. **IMPACT OF FASCIOLA HEPATICA INFECTION AND TRICLABENDAZOLE TREATMENT ON HUMORAL IMMUNE RESPONSE AND VACCINE EFFICACY IN CATTLE**

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Fasciola hepatica is a trematode parasite of sig-

nificant veterinary and public health importance, causing economic losses in livestock due to liver damage, weight loss, and reduced milk production. Although triclabendazole (TCZ) is available for treatment, it does not prevent the disease or reinfection. Infected animals exhibit strong immunoregulation, increasing susceptibility to secondary infections and altering vaccine-induced antibody responses. This study investigates the humoral immune response in cattle infected with *F. hepatica* at different stages of infection and evaluates the effect of TCZ treatment on this response. It also examines how fasciolosis affects the antibody response induced by bacterial vaccines during early and chronic infection stages. Experimental infections in steers were conducted, with fecal and plasma samples collected at various intervals. The results showed a decrease in parasite-specific antibody avidity during infection, with a significant drop post-TCZ treatment. Furthermore, *F. hepatica* infection compromised the antibody response to bacterial vaccines. This study underscores the need for further research on the impact of fasciolosis and its treatment on livestock vaccination efficacy.

202. 049. **PEPTIDES DMS-DA6 AND DRS-DA2N HAVE IMMUNOMODULATORY EFFECTS IN A MOUSE MODEL OF ACTINOMICETOMA BY N. BRASILIENSIS**

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Actinomycetoma is an infectious disease recognized by the World Health Organization as a neglected tropical disease. In the Americas, the most common etiologic agent is the Gram-positive bacterium *Nocardia brasiliensis*. Current treatment is prolonged, toxic and resistant strains have emerged. A promising solution is the use of host defense peptides, which are recognized for their bactericidal activity and ability to control infection by modulating the host immune system. Recently, two new antimicrobial peptides have been described. DMS-DA6 and DRS-DA2N, isolated from the skin of the Mexican frog *Pachymedusa dacnicolor*, are members of the dermaseptin family. These peptides have been demonstrated to exhibit remarkable bactericidal activity against Gram-positive bacteria, and the DRS- DA2N peptide also exerts an immunosuppressive effect by regulating the inflammatory cell pool. The objective of this study is to assess the *in vivo* immunomodulatory and antibacterial effects of DMS-DA6 and DRS-DA2N peptides in a model of actinomycetoma by *N. brasiliensis*. Two distinct treatments were administered over a three-week period. The first treatment utilized solely DMS-DA6, while the second treatment combined DMS-DA6 with DRS-DA2N. Following completion of the treatment period, there was a notable reduction in the size of the infected area, accompanied by a significant decline in the bacterial load. Furthermore, flow cytometry analysis demonstrated that both treatments modify leukocyte populations locally and systemically. This resulted in a decrease in the number of monocytes/macrophages and CD4+ and CD8+ T lymphocytes in the blood and spleen. In contrast, the number of CD8+ and CD4+ T lymphocytes increased in the infected plantar pad. Furthermore, it was observed that the levels of pro- and anti-inflammatory cytokines quantified in serum by multiplex significantly decreased upon administration of the peptides. These results suggest that the peptides DMS-DA6 and DRS-DA2N are a promising alternative approach and a valuable addition to the current anti-infective strategy.

203. 063. **MICROPLASTICS' EFFECT ON PERIPHERAL BLOOD MONONUCLEAR CELLS IN BACTERIAL INFECTIONS**

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Background: Microplastics are a concern for the environment and the health of humans. In the last two decades, some studies demonstrated that microparticles derived from commonly used plastics such as bottles and food packaging can be found in almost any tissue in the human body and may not be as innocuous as initially thought. That is why the studies of microplastic immunotoxicity have been increasing recently. Recent studies have shown the presence of microplastic particles in human peripheral blood; however, their potential impact on immune cells has remained elusive.
Objective: To investigate the response of peripheral blood mononuclear cells (PBMC) to polyethene terephthalate (PET) microplastics in the context of bacterial infections. The hypothesis was that microplastics affects the secretion of cytokines in bacterial infections. **Methods:** Mea-

surement of the viability of cells and levels of cytokines such as IL-1β, TNF-α, IL-6 and IL-10 were performed, and the results were compared with those found in the literature. **Results:** Data suggest that PET microplastics can influence on the secretion of inflammatory cytokines in different bacterial infections. In infections with *Salmonella sp*. there were a significant ($p<0.05$) increase of TNF-α and decrease of IL-6; there were not significant effect of microplastics in *Escherichia coli* and gram-positive bacteria. **Conclusion:** Results are highlighting the influence of environmental pollutants in infectious diseases. Future studies should focus on investigating the impact of microplastics with different chemistry and size ranges on a more significant number of donors to better understand the potential impact of exposure to microplastics.

204. 064. **TIM-3 AND PD-1 EXHAUSTION MARKERS ON T LYMPHOCYTES IN AN EXPERIMENTAL ACTINOMYCETOMA MODELTIM-3 AND PD-1 EXHAUSTION MARKERS ON T LYMPHOCYTES IN AN EXPERIMENTAL ACTINOMYCETOMA MODEL**

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In Mexico, *Nocardia brasiliensis* is responsible for 85% of the cases of actinomycetoma, an infectious disease of an inflammatory, destructive and disabling nature. The immunological events that occur during *N. brasiliensis* infection have not yet been elucidated in humans or experimental models. PD-1 and TIM-3 belong to the family of molecules that mediate the negative regulation of T cells; the increased and sustained co-expression of these markers has been widely related to the depleted T cell phenotype. In the present work, Balb/c mice infected in the foot pad with *N. brasiliensis* were used and the kinetics of expression of these markers in T lymphocytes was evaluated by flow cytometry. Lymphoid organs such as the popliteal node and spleen and the infected tissue were studied during the acute and chronic phase of the infection. The results obtained show that the coexpression of PD-1 and TIM-3 increased in T lymphocytes in the chronic phase of the infection compared to the acute phase, occurring both in the studied lymphoid organs and in the infected tissue; therefore, it is suggested that the expression of both markers during the chronic phase could be associated with the depleted T cell phenotype, thus contributing to the persistence of the infection. The biological blockade of depletion markers has a therapeutic potential in patients.

205. 066. **SELECTIVE ENHANCEMENT OF MAST CELL INFLAMMATORY RESPONSE BY IL-33: NEW PERSPECTIVES ON ANTIMICROBIAL ACTIVITY**

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Mast cells (MC) are key immune cells found throughout the body, especially in areas in contact with the external environment. These cells can recognize various stimuli, including alarmins and microorganisms, through a diverse range of receptors. IL-33, an alarmin released during tissue damage, is recognized by immune cells, including MC. While IL-33 enhances allergic responses in MC, its effects on other activation pathways remain unclear. Because MC play an essential response in the early response to infection, the aims of this s
tudy was to evaluate the impact of IL-33 on MC inflammatory activation in response to specific TLR ligands, *Escherichia coli* and *Mycobacterium bovis* BCG bacteria. To conduct the study, MC derived from the bone marrow (BMMC) of C57BL/6 mice were cultured in a medium supplemented with IL-3 and SCF for 6-8 weeks. These cells were pretreated with IL-33 and then stimulated with the TLR ligands LPS, Pam3CSK4 or bacteria. BMMC activation was assessed by measuring degranulation through beta-hexosaminidase release and the production of cytokines, chemokines, and prostaglandin D2 using ELISA assays. Notably, we observed a significant increase in

pro-inflammatory cytokine production in BMMC pretreated with IL-33 and stimulated with LPS or *E. coli*. However, this effect was not observed with Pam3CSK4 or *M. bovis* BCG. Interestingly, there was no impact on mast cell degranulation or prostaglandin D2 (PGD2) production under any condition. These findings indicate a selective enhancement of mast cell inflammatory responses by IL-33, providing new insights into its role in antimicrobial activity and potential therapeutic applications.

206. 068. **PURINERGIC SIGNALING AS A POTENTIAL TARGET TO IMPROVE IMMUNIZATION IN COVID-19**

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Immunization plays a crucial role in preventing viral infections. However, vaccine efficacy can vary depending on factors such as the pathogen's nature, vaccine type, and individual immune response. To improve immunization strategies, understanding and manipulating underlying immunological mechanisms that enhance vaccine responses are essential. This study investigated optimal immunization conditions to optimize the generation and maintenance of tissue-specific resident memory T cells (TRMs) specific to SARS-CoV-2 in the lung. C57BL/6 mice were immunized with inactivated SARS-CoV-2 via intravenous and intranasal routes (IV+IN) or intranasal only (IN). IV+IN immunization involved an IV dose on day 0 and three IN doses on days 7, 10, and 13. On day 46, lung tissues were analyzed. To explore eATP interaction during immunization, the same strategy was repeated with the addition of 4.5 mM BzATP to intranasal doses. Fluorescent anti-CD45 intravenous antibodies and anti-ARTC2 nanoantibodies were used to differentiate cells in the lung parenchyma and vasculature, preventing NAD- and ATP-induced cell death. CD4+ and CD8+ T cell responses in the lung parenchyma were evaluated by flow cytometry. IV+IN immunization induced a robust population of activated lung cells expressing high levels of the extracellular receptor P2RX7, crucial for TRM generation and maintenance. However, adding eATP to inactivated SARS-CoV-2 immunization resulted in a reduced response, possibly due to high ATP doses. Understanding immune responses to infection-induced damage allows more effective manipulation of TRM generation and maintenance, contributing to tissue-specific memory development. This study provides valuable insights for optimizing vaccine strategies against viral infections.

207. 069. **DIFFERENT TYPES OF PROPOLIS IMMUNOMODULATE NEUTROPHIL RESPONSE AND HAVE ANTIMICROBIAL EFFECT ON PARACOCCIDIOIDES BRASILIENSIS AND PARACOCCIDIOIDES LUTZII**

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Paracoccidioidomycosis is a severe systemic mycosis caused fungi belonging to the Paracoccidioides spp. complex. The big challenge in treating mycosis is to reduce the time spent using antifungal medications. There are 13 types of propolis already identified and red propolis is a new type that has unique chemical and biological profiles. Our objective was to evaluate the antifungal and immunostimulatory activity of propolis from different sources against *Paracoccidioides* spp. The tests were conducted as follows: analysis of the chemical composition of brown (PM), green (PV) and red propolis (PVM), *in vitro* verification of cytotoxicity on host cells, evaluation of fungicidal activity and quantification of reactive oxygen species (ROS), hydrogen peroxide(H_2O_2), catalase(CAT), and total (PTs). The chemical composition indicated contents of 0.42, 1.0 and 0.44g of flavonoids and 3.50, 4.01 and 3.19g of phenolic compounds in the dry extract of PM, PV and PVM respectively. The *in vitro* results showed that all types of propolis presented antifungal activity against the strains of *Paracoccidioides* spp. tested. None of the propolis employed had cytotoxic effect on host cells. *Ex vivo* tests showed that none of the propolis used affected cell viability at the concentrations used, but all of them reduced the influx of neutrophils to the infection site. The data showed that all propolis showed increased activity, as verified by the following parameters:

production of CAT, H_2O_2, ROS and PTs when tested alone or in combination. A remarkable fungicidal effect was also detected, mainly of PVM. These results suggest that in the future, propolis may be combined with well-established drugs to shorten the treatment of paracoccidioidomycosis.

208. 070. CHANGES IN CELLULAR PROFILE AND IN IL-4 AND IFN-GAMMA LEVELS INDUCED BY DIFFERENT DOSES OF FLUCONAZOLE IN A MURINE MODEL OF PARACOCCIDIOIDOMYCOSIS

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Fungi of the genus *Paracoccidioides* spp. are responsible for causing paracoccidioidomycosis (PCM), a severe systemic granulomatous mycosis. The triazole antifungal Fluconazole (FluZ) is generally used in the treatment of PCM in cases when there is contraindication to traditional treatment. The present study sought to evaluate the effect of FluZ on leukocytes. For this purpose, female Swiss mice were infected with *P. lutzii* via subcutaneous air pouch. From the 5th day of infection, mice were treated with FluZ at concentrations of 1 and 2mg/mL. On the 8th day, the exudate present in the air pouch was collected and the following parameters were analysed in the material obtained: absolute and relative number of viable cells, mitochondrial activity, microbicidal activity of phagocytes and content of the cytokines IL-4, IL-17, IL-12, TNF-alpha and IFN-gamma. The 1mg/mL dose of FluZ reduced the absolute number of cells present in the air pouch. On the other hand, at a dose of 2mg/mL there was no difference from the control, only infected group. Both doses were shown to metabolically activate cells by the mitochondrial dehydrogenase test, but the concentration of 2mg/mL resulted in more activation. The cellular profile in the air pouch was altered by both doses of treatment, with a reduction in neutrophils compensated by an increase in lymphocytes. We also observed that FluZ did not affect phagocyte activity. Regarding cytokines, both doses tested did not affect the IL-4 content and increased that of IFN-gamma. The 2mg/mL dose significantly increased the levels of IL-17. Interestingly, the 1mg/mL dose of FluZ increased TNF-alpha and reduced IL-12. Therefore, the FluZ doses demonstrated different behavior in terms of cytokine production. The change in the cellular profile strongly suggest an anti-inflammatory effect of FluZ. This activity as well as its activation of cells make FluZ a promising therapeutic option for PCM in the future.

209. 071. NEUTROPHILS SUPPORT BRUCELLA DISPERSAL WITH REDUCED IMMUNE RECOGNITION

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Neutrophils (PMNs) are the first line of defense against bacteria entering the body. However, it has been shown that *Brucella* induces low PMN activation and survive within these leukocytes by resisting their microbicidal mechanisms. *Brucella* also induce the premature cell death of PMNs, which release chemokines and express “eat me” signals. These PMNs are then phagocyted by mononuclear cells where *Brucella* replicate. This evidence suggests that PMNs may behave as vehicles protecting *Brucella* from immune recognition, favoring their dispersion to the target organs. To test this hypothesis, we analyzed the course of infection in mice intraperitoneally infected with *B. abortus* alone (Ba) or with *Brucella*-infected PMNs (Ba-PMN). We evaluated bacterial loads, histopathological analyzes, cytokine production in serum, anti-*Brucella* antibody titers, and hematological parameters. We observed that mice infected with Ba-PMN had lower bacterial loads in the spleen and bone marrow at seven days of post-infection compared to Ba. The bacterial load then became equivalent at 30 days post-infection. The pathological index demonstrated a similar trend to the bacterial load. Comparably, Ba-PMN infected mice showed less IFN-gamma and IL-6 at the beginning of the infection than the Ba group but with similar concentrations at the end of the experiment. Ba-PMN infected mice also showed fewer anti-*Brucella* antibody titers at day 30 than the Ba group. No significant differences were observed between infected groups in the hematological values at 30 days. Despite both groups reaching similar bacterial loads by day 30, the bacterial loads and the immunological parameter were lower at the beginning of the infection in the Ba-PMN group. We conclude that the course of infection in the Ba-PMN group was stealthier than in the Ba group. Despite the slower course of *Brucella* infection in the Ba-PMN, PMNs supported their dispersion to a similar extent but with reduced immune recognition.

210. 085. **EFFECT OF THE COMBINATION OF FENOFIBRATE AND LINEZOLID, ON ACTINOMYCETOMA BY NOCARDIA BRASILIENSIS IN BALB/C MICE**

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Actinomycetoma is an infectious, chronic, inflammatory disease, mainly caused by *N. brasiliensis*. Treatment with Amikacin and Trimetroprim is the most widely used and needs to be administered for years. Angiogenesis could contribute to the persistence and spread of the infection, while inflammation prevents the elimination of the infectious agent. Fenofibrate is a widely used drug to reduce triglyceride levels as a PPAR agonist, it has great anti-inflammatory and anti-angiogenic effect; therefore, its use, combined with an antibiotic such as Linezolid could be an alternative treatment for actinomycetoma. The purpose of this study is to evaluate the effect of the combination of fenofibrate and linezolid as a treatment of actinomycetoma by *N. brasiliensis* in an experimental model. The effect of the drugs alone or in combination was evaluated in BALB/C. mice with *N. brasiliensis* actinomycetoma, and the clinical and histopathological evolution of the lesions, angiogenesis, IL-1β and IL-6 production, VEGF and expression of pro- and anti- angiogenic factors (VEGF, COX-2 and TSP-1) were analyzed. Improvement was observed in all treatments. The combination of Linezolid/Fenofibrate decreased the percentage of IL-1β, as well as the PMN infiltrate, the presence of VEGF in the tissue and the expression of the mRNA thereof; these effects were greater compared to Linezolid and Fenofibrate alone. The treatment with Linezolid decreased the percentage of foam cells in the tissue more effectively, compared to the combination and Fenofibrate alone, although the latter induced greater expression of TSP-1 in the tissue at day 7 of the treatment. All treatments significantly decreased COX-2 levels.The combination of Fenofibrate/Linezolid, has a synergistic effect by more effectively decreasing inflammation, lesions and the angiogenic process, in the actinomycetoma and offers a therapeutic potential.

211. 087. **EPIDEMIOLOGICAL PROFILE OF DENGUE VIRUS INFECTION IN GOIÁS, BRAZIL, DURING THE PERIOD 2018-2024**

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Background: Dengue fever is a neglected tropical disease caused by one of the 4 serotypes of the dengue virus (DENV) and transmitted to humans by female *Aedes* mosquitoes. Symptoms range from mild fever to severe hemorrhagic fever and shock syndrome. As a neurotropic virus, the most frequent neurological complications are encephalitis (direct viral invasion), encephalopathy (cytokine mediated), seizures, and Guillain-Barré Syndrome. In recent years, there have been outbreaks in Goiás, Brazil and around the world. Objectives: Thus, we aimed to evaluate dengue incidence in Goiás during 2018-2024, verifying whether some risk factors may interfere with this disease prognosis. Method: This was a retrospective epidemiological work, based on data collected from the Brazilian Informatics Department of the Unified Health System (DATASUS), Ministry of Health and epidemiological bulletins. There was no need for an opinion from the Ethics Committee in Research, as secondary data are public. Results: The data show that 913,371 cases of dengue were reported in Goiás, of which 526,487 (57.64%) in the brown/black, 159,586 (17.47%) in white, 11,078 (1.21%) in yellow and 2000 (0.22%) in indigenous populations. One can also observe that 2024 was the year with the highest case notification frequency (293,698), followed by 2022 (208,666), increasing 312.39%, 228.37%, and 505,00%, compared to 2023 (67,861), 2021 (61,438), and 2020 (58,146) respectively. It draws attention to the fact that in 2024, these data refer only up to the beginning of June, surpassing all-time indices. Conclusion: These data suggest that the pandemic period (2020-2021) and socioeconomical risk factors affected the number of cases notified and the public policies for preventing the mosquito spread. Thus, these results show that there is a demand for efficient public policies to combat the mosquito vector, such as adequate waste disposal and vaccination campaigns, among others.

212. 088. **SHARED ROLES BETWEEN ECHINOCOCCUS GRANULOSUS ANTIGEN B AND VERTEBRATES HDL: IMMUNOREGULATION AND LIPID ACQUISITION PROPERTIES**

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The larva of *Echinococcus granulosus* causes cystic echinococcosis, a chronic viscera infection -mainly the liver- implying a tight control of host immunity. Its location in a medium rich in nutrients shaped parasite's metabolism, losing de novo fatty acid and cholesterol synthesis pathways together with the expression of proteins capable of capturing/transporting essential lipids. Antigen B (EgAgB), the main larva lipoprotein, is a member of the cestode-specific hydrophobic ligand-binding protein family, being exported through unknown mechanisms to host tissues. EgAgB resembles vertebrates HDL in molecular size, lipid:protein ratio, lipids heterogeneity and α-helix predominance on its apolipoproteins. Moreover, EgAgB and HDL share anti-inflammatory properties on innate cells. This work goes deeper into EgAgB's biological activities, in comparison with HDL. Results showed that native EgAgB and HDL modulate LPS-driven macrophage activation, with lower EgAgB concentration needed to reach similar IL-6 inhibition. In competition assays, both lipoproteins decreased LPS binding to macrophages, suggesting EgAgB interacts with LPS interfering with its cellular recognition and inflammatory consequences, as HDL does. Besides, direct binding analysis revealed that EgAgB bound LPS in a larger extent than HDL, interaction favoured by the presence of LBP. An EgAgB scavenger activity of enteric LPS that reaches the liver could contribute to decrease harmful consequences of LPS-driven inflammation in the parasite vicinity, a physiological role that HDL plays in this organ. EgAgB's ability to scavenger other PAMPs/DAMPs requires further studies. On the other hand, since EgAgB carries host cholesterol and plasma lipoproteins can exchange lipids, we analysed EgAgB's ability to uptake cholesterol from HDL. EgAgB captured fluorescent cholesterol from previously loaded HDL, suggesting additional interactions between EgAgB and HDL for parasite cholesterol acquisition. Altogether, results support a dual role of EgAgB in *E. granulosus* biology, contacting host components to acquire essential lipids while contributing to protecting the parasite from host inflammation.

213.090. TWO FUNCTIONS, ONE PARASITE LIPOPROTEIN: ANTIGEN B UPTAKES CHOLESTEROL AND ACTS AS AN EFFICIENT LPS-SCAVENGER

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The larvae (hydatid) of *Echinococcus granulosus* s.l. grows within the host´s viscera causing a chronic infection. This highlights an excellent parasite adaptation to its hosts, involving a tight modulation of the immune response with several mechanisms likely involved. An *E. granulosus* lipoprotein, called antigen B (EgAgB), was postulated as immunomodulator because of its capacity to interfere with innate cell activation *in vitro* and *in vivo*. EgAgB belongs to a cestode-specific family of hydrophobic ligands binding proteins, having putative participation in acquiring lipids not synthesized by *Echinococcus (*cholesterol and fatty acids). EgAgB physicochemical characterization (size, lipid/protein ratio, apolipoprotein secondary structure) revealed similarities to HDL, described as a plasma lipophilic PAMP scavenger and anti-inflammatory lipoprotein due to its ability to remove cholesterol from innate cells. To address EgAgB mechanisms involved in innate cell modulation, we compare *in vitro* EgAgB and HDL effects on dendritic cells (BMDC) activation. EgAgB was significantly more efficient

in inhibiting LPS-induced IL6/IL12 secretion on BMDC than HDL. Unlike HDL, EgAgB did not alter LTA-induced cytokine secretion, revealing a specificity for LPS interference. Of note, EgAgB diminished LPS-induced TLR4 dimerization, an early step of TLR4 activation pathway, and bound equally to TLR4KO and wild-type BMDC suggesting it controls activation in a receptor independent manner, previous to LPS-TLR4 interaction. Additionally, EgAgB inhibited LPS binding to BMDC, possibly neutralizing LPS in the milieu as HDL3 does. A direct interaction between EgAgB and LPS was observed by an ELISA-like assay, supporting LPS neutralization might contribute to EgAgB´s modulatory effects on innate cells. Whether EgAgB neutralizes/carries other immune-relevant molecules deserves analysis. Notably, EgAgB removed cholesterol from macrophages and hepatocytes, by an SR-B1 and ABCA-1 independent mechanism (unaltered by specific inhibitors), suggesting an efficient passive diffusion mechanism. Further studies are needed to elucidate if EgAgB's ability to uptake cellular cholesterol impact innate cell activation.

214. 101. **HIV LATENCY REVERSAL IN A MYELOID MODEL CELL LINE: IMPACT OF SARS-COV-2 DIRECT AND BYSTANDER EFFECTS**

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Background: People living with HIV (PLWH) are at higher risk for severe COVID-19. A key challenge in curing HIV is the reservoir of infected cells resistant to combination antiretroviral therapy (cART). The effect of SARS-CoV-2 on the HIV reservoir in PLWH remains unclear, but pro-inflammatory cytokines from SARS-CoV-2 may reactivate HIV- 1 latency in myeloid cells. **Objectives:** To determine if SARS-CoV-2 reactivates HIV-1 latency directly or via cytokines from infected macrophages using a myeloid in vitro model (U1). **Methods**: HIV latency reversion was assessed by measuring intracellular HIV-p24 expression via flow cytometry. U1 cells were exposed to SARS-CoV-2 ancestral and BA.5 variants (MOI=0.1) for up to 3 days. For indirect effects, MDM were exposed to the ancestral SARS-CoV-2 strain (MOI=0.1), and these conditioned media (CM) were used to evaluate latency reversion in U1 cells. TNF-α neutralization was performed using Infliximab. **Results**: Direct exposure of U1 cells to SARS-CoV-2 (ancestral and BA.5) strains slightly increased HIV-p24 expression (0.41±0.25%, 2.4±0.6%, and 2.7±0.5%). U1 cells did not sustain productive SARS-CoV-2 infection (viral RNA in supernatants measured by qPCR). Macrophages exposed to SARS-CoV-2 showed an M1 (CD80+, 76.8±7.1%) to M2 (CD206+, 95.0±3.5%) profile shift over 72 hours, with corresponding changes in TNF- α levels (760.4±238.4 vs. 228.7±226.7 pg/mL, respectively). Conditioned media from MDM exposed for 24 hours exhibited the highest latency reversal capacity (57.4±17.1%), compared to 16.2±6.2% after 72 hours. Neutralizing TNF-α with Infliximab significantly decreased HIV latency reversal from 33.7±0.4% to 4.9±0.3%. **Conclusions**: SARS-CoV-2 can slightly reverse HIV latency directly, but primarily via a bystander mechanism through a pro-inflammatory microenvironment driven by TNF-α from infected macrophages. This study highlights the complex interplay between SARS-CoV-2 and HIV, emphasizing the role of pro-inflammatory mediators in enhancing HIV replication.

215. 102. **DIETHYLCARBAMAZINE REGULATES CYTOKINE PRODUCTION AND REDUCES THE VIRAL TITER IN EPITHELIAL CELLS INFECTED BY THE INFLUENZA A (H1N1)PDM09 VIRUS.**

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Background: Influenza is a viral infection with significant pandemic potential, and theimmune response, involving respiratory epithelial cells, is crucial for resolving the disease.Insomecases,thecytokinestormisresponsiblefortissuedamageandbarrierdysfunction.Diethylcarbamazine(-DEC),adrugwithimmunomodulatoryeffects,hass-

hownanti-inflammatory and antifibrotic properties in different models. Nevertheless, DEC's activity inviral respiratory infections remains unknown. **Objective:** The aim of this study was toanalyzeDEC'seffectinamodelofrespiratoryepithelialcellsinfectedwithInfluenzaAH1N1pdm09 virus (IAV). **Methods:** A549 cells were cultured, infected with IAV, and then treatedwith DEC. Cytokine levels (TNF-α, IL-1β, IL-6, IL-8, IL-10, IL-15, and RANTES), geneexpression (RIG-I, TLR3, MYD88, NLRP3, NF-κB, IFN-β, and IFN-λ) and viral load weredetermined at 24 and 48 hours. Data normality was assessed, followed by ANOVA withTukey's post hoc for parametric data and Kruskal-Wallis with Dunn's post hoc for non-parametric data, using GraphPad Prism 9. **Results:** IFN-λ1 gene expression levels weresimilar across all groups (*P>0.05*). IFN-β gene expression were increased at 24 hours(*P<0.05*) but not at 48 hours in infected groups treated with DEC (*P>0.05*). At 24 hours, IL-8 secretion was significantly higher in the VIA + DEC20 group compared to the MOCK (P≤0.05),howeverat48hours,nosignificantdifferenceswereobservedamongstudiedgroups(*P>0.05*). The group of IAV-infected cells treated with the lowest dose of DEC had a lowerviral titer compared to the infection group (P≤ 0.05). **Conclusion:** DEC maintains IFN-λ1expression, regulates levels of IFN-β and IL-8, and reduces viral load in epithelial cellsinfectedwiththe2009H1N1pandemicInfluenzaAvirus.

216. 104. EFFECT OF HUMAN METAPNEUMOVIRUS INFECTION ON THE EXPRESSION OF APOPTOTIC AND CO-STIMULATORY PROTEINS IN MYELOID IMMUNE CELLS.

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Human Metapneumovirus (hMPV) is a respiratory virus that primarily affects children, the elderly, and immunocompromised individuals. This virus mainly infects the epithelial cells of the lungs. Typically, epithelial barriers respond to viral infections by activating type 1 interferon pathways, which are associated with the release of cytokines that promote T cell differentiation towards the Th1 subtype, thereby enhancing inflammation and antiviral responses. However, the direct interaction between hMPV and innate immune system cells has not been fully elucidated. Macrophages and dendritic cells play a crucial role in processing and presenting antigens to T cells, which involves co-stimulatory molecule expression. Moreover, it has been reported that various respiratory viruses can modulate the expression of anti-apoptotic proteins of the Bcl-2 family in antigen-presenting cells, suggesting that these cells may also be targeted by hMPV infection. This study demonstrates that hMPV can infect cells of the innate immune system, leading to an increase in the expression of anti-apoptotic proteins and a decrease in the expression of co-stimulatory molecules. *In vitro* assays revealed an upregulation of the anti-apoptotic proteins Mcl-1 and Bcl-2, and a downregulation of the pro-apoptotic protein Bax, as determined by qPCR, assessing hMPV-infection over RAW264.7 cell line, and primary cultures of both bone marrow-derived macrophages and dendritic cells from C57BL/6 mice. Additionally, there was a reduction in the expression of co-stimulatory molecules such as CD86 and decreased secretion of cytokines, including IFN-γ, IL-6, and IL-10. These findings provide valuable insights into potential mechanisms by which hMPV may evade the immune system.

217. 109. THE SECRETORY IGA IN SALIVA OF COVID19- CONVALESCENT PATIENTS IS ASSOCIATED WITH LACK OF REINFECTION FOR AT LEAST ONE YEAR. VACCINE DEVELOPMENT PERSPECTIVES

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The anti-SARS-CoV-2 vaccines approved in Argentina are administered intramuscularly, triggering a systemic immune response but does not elicit a robust mucosal immunity response. Given that SARS-CoV-2 is a virus whose entry point is respiratory mucosa, we wonder if mucosal immune response, particularly secretory IgA, may have a protective effect against infection. To assess this question, we developed an ELISA

to measure levels of anti-RBD-SARS-CoV-2 IgA (RBD: receptor binding domain) in saliva of vaccinated and convalescent patients, assuming a good correlation with neutralizing IgA levels, and then analyze the risks of infection/reinfection. 50 patients were recruited, of which 45 met the inclusion criteria either as cases (35 convalescent patients between 28 and 35 days post-symptom onset data) or as controls (10 patients who remained symptom-free since the start of the pandemic with a negative test for SARS-CoV-2), having received at least two doses of vaccines at the time of saliva sample collection (December 2021-February 2022). Our findings revealed that 14% (5/35) of the convalescent group were negative for IgAs, while 86% (30/35) had detectable levels of IgAs. On the other hand, within the second group, we observed that 50% (5/10) of the controls had non-detectable anti-RBD-SARS-CoV-2 IgAs. Kaplan-Meier analysis showed that the risk of infection or reinfection is significantly lower in patients with anti-RBD-SARS-CoV-2 IgAs in saliva (p=0.0318). These results suggest that the infection/reinfection risk is significantly lower in individuals with Mucosal Immune Response, reflected in the presence of anti-SARS-CoV-2 IgAs. Our results indicate that a vaccine designed to stimulate the Mucosa-Associated Immune System may be the pathway to eradicating SARS-CoV-2.

218. 111. **DEVELOPMENT OF A IMMUNOCOMPETENT DERMAL MURINE SPHEROID MODEL FOR STUDIES ON CUTANEOUS LEISHMANIASIS**

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Cutaneous leishmaniasis (CL) is an infection caused by the protozoan parasite *Leishmania* and the vector is the female sandfly. According to the World Health Organization (WHO), the disease affects over 12 million people globally, with 2 million new cases arising annually, and is classified as an emerging, uncontrolled and neglected disease in tropical contexts. Endemic in almost 100 countries, CL represents a risk to approximately 350 million people. The disease leads to the development of skin lesions after inoculation of the parasite into the skin, and the nature of the immunity required to control it is still uncertain. Biopsies of the lesions may help in understanding the immune response, but are limited by invasiveness and require dermatological expertise. To overcome these challenges, 3D skin culture models are suggested, such as spheroids. Therefore, we sought to develop and test an immunocompetent dermal spheroid model for studies on CL. Murine fibroblasts of the NCTC clone 929 (L929) and macrophages of the J774 1.6 (J774) lines were cultured to form aggregates using the *Hanging Drop* technique, and were infected with *Leishmania amazonensis* marked with CellTrace CFSE Cell Proliferation Kit (Invitrogen). Finally, nitric oxide (NO) levels were measured using the Griess method. Efficient compaction was observed using the L929 and J774 lines. The infected spheroids showed greater instability in conformation when compared to the uninfected ones, demonstrating that the infection may influence the interaction of components of the extracellular matrix and cells. The infection was successful and confirmed by fluorescence microscopy. However, the production of NO, important in the immune response, was insufficient. The model looks promising for the study of interactions between host cells, parasites and extracellular matrix in LC. Further studies in our research group are underway to better standardize the model.

219. 113. **PROFILING THE HLA-I AND HLA-II IMMUNOPEPTIDOME OF T. CRUZI ANTIGENS USING MASS SPECTROMETRY AND BIOINFORMATICS**

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The study of T cells results crucial to the understanding of the immune response in Chagas disease. However, many challenges are faced when attempting to identify T cell epitopes for diagnostic or preventive purposes. In this context, our work aims to evaluate the immunogenicity of *T. cruzi* antigens identified by an immunopeptidomics approach, in samples from chronic Chagas disease patients. To this end, we profiled the repertoire of HLA-bound peptides presented by THP-1 macrophages infected or not with *T. cruzi* by MS and predicted their binding with algorithms based on artificial neural network. Results showed no difference in length between *T. cruzi* and uninfected host ligands, thus suggesting that the parasite does not alter antigen processing and presentation machinery in the host cell. Analysis of antigens allowed us to identify 64 *T. cruzi* encoded ligands originating from 34 proteins. For these source proteins, we predicted the binding of peptides extracted for HLAs prevalent in endemic areas or worldwide and selected those with optimal allelic and antigenic coverage. These peptides and those identified by MS, were selected for *in vitro* screening and randomized in five equal-sized pools to challenge PBMC samples from chronic Chagas disease patients, using IFN-gamma secretion as readout. Until now, peptide pool-specific responses were detected in three out of 24 analyzed patients who also responded to the *T. cruzi* lysate, suggesting an association of peptide reactivity to memory response. As far as we know, this study represents the most comprehensive immunopeptidomic dataset available for *T. cruzi* to date. Despite the limited size of our universe of peptides, we were able to distinguish a pool of sequences that contain *T. cruzi* epitopes. Thus, this study demonstrates that the developed pipeline can be successfully used to identify *T. cruzi* epitopes recognized by T cells from chronically infected patients.

220. 132. **SKIN ANTIFUNGAL IMMUNITY: IN VIVO ROLE OF NEUTROPHILS IN CONTROLLING NANNIZIA GYPSEA EXPERIMENTAL DERMATOPHYTOSIS**

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Fungal skin infections significantly contribute to the global burden of disease. However, the understanding of cutaneous immunity against dermatophytes remains limited. Our previous research established a model of epicutaneous infection using the human pathogen *Nannizzia gypsea* in C57BL/6 mice, highlighting the critical role of IL-17RA signaling in anti-dermatophyte defenses. Notably, we demonstrated that neutrophil recruitment and their functions are uncoupled from IL-17-mediated immunity. This study investigates the role of neutrophils *in vivo* during *N. gypsea* dermatophytosis in C57BL/6 WT and IL-17RAKO mice.

Mice were epicutaneously infected on the back skin with a suspension of *N. gypsea* (OD=1.00). At 24, 48, 72 h, and 6 days post-infection (dpi), the skin was removed and treated with trypsin (2 h, 37°C) to obtaining epidermal cell suspensions for fungal load quantification (CFU/g skin), FACS analysis and reactive oxygen species (ROS) production. Skin section from infected were also analyzed by histopatology and immunofluorescence. For neutrophil depletion, a group of mice were intraperitoneally injected with anti-Ly6G (AI8 clone, BioXcell) or anti-Gr1 (clone RB6-8C5 purified from hybridoma culture supernatant) and isotype controls on -1, 0, and 2 dpi. Fungal load, skin cell populations, and histopathology were analyzed at 3 dpi. *N. gypsea*-infected IL-17RA-KO mice exhibited significantly higher neutrophil skin recruitment ($p<0.04$, 48h; $p<0.0023$, 72h; $p<0.0001$, 6 dpi) and ROS production throughout the infection compared to WT mice. Immunofluorescence analysis revealed significantly increased neutrophils recruitment in the epidermis and stratum corneum of IL-17RAKO mice compared to WT mice. Furthermore, neutrophil depletion after anti-Gr1 or anti-Ly6G treatment did not affect infection susceptibility in WT mice but anti-Gr1 administration increased fungal load in IL-17RAKO mice ($p<0.0093$, 3 dpi). This results highlights the importance of neutrophils in early antifungal defense, particularly in the absence of IL-17RA signaling.

221. 136. **CELLULAR EXHAUSTION MARKERS ANALYSES IN T LYMPHOCYTES FROM PATIENTS WITH CUTANEOUS LEISHMANIASIS**

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Background: Cutaneous leishmaniasis (CL) is the most prevalent form of Leishmaniasis, a public health issue. Literature shows that immune activation can induce the expression of co- inhibitory molecules, leading to its reduced effector function. These co-inhibitory pathways, which are not well understood, may be associated with the chronicity of CL. **Objective:** Analyze cellular exhaustion markers in patients. **Methods:** Blood samples were collected from 9 patients with active CL before treatment (BT), 15 after treatment (AT), and 16 controls (HC) to obtain PBMCs. This was followed labeling with anti-CD3, CD4, CD8, FOXP3, PD1, TIGIT, and TIM-3 antibodies. Cells were acquired using a FACSARIAIII and data were analyzed with FlowJo. **Results:** TIGIT expression on CD4+ cells was significantly higher in the BT group compared to the AT and the HC groups. PD1 expression was significantly elevated in both the BT and AT groups compared to HC group. The expression of PD1+TIM3- on CD4+ cells was higher in the BT and AT groups compared to the HC group. The BT group showed significantly higher levels of PD1+TIM3+TIGIT+ compared to the HC and AT groups. In Treg cells, TIGIT was significantly elevated in the BT group compared to the HC and AT groups, while the HC group showed higher expression of TIM-3 and PD1+TIM3+ compared to the BT group. PD1+TIM3- expression was significantly higher in the BT group compared to the HC group. PD1+TIM3+TIGIT- expression was higher in the AT group compared to the BT and HC groups. In CD8+ cells, TIGIT was more expressed in the AT group compared to the HC group. **Conclusion:** The expression of exhaustion markers by patients, such as PD1, TIM-3, and TIGIT, suggest their role in modulating the immune response and disease progression.These findings highlights the importance of investigating co-inhibitory pathways in CL for the development of new therapies.

222. 138. BRUCELLA ABORTUS DNA MODULATES MSC DIFFERENTIATION INTO OSTEOBLASTS AND ADIPOCYTES INDUCING A PROINFLAMMATORY PROFILE

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The most common manifestation of active brucellosis in humans is osteoarticular injury. The differentiation of mesenchymal stem cells (MSCs) into adipocytes or osteoblasts is regulated by mechanisms that promote cell fate toward one lineage while repressing the other.
MSCs were infected with *Brucella abortus* (*Ba*) at a multiplicity of infection (MOI) of 100. Then, the culture medium was replaced with either osteoblast or adipocyte differentiation medium. Osteoblast differentiation was assessed using Alizarin Red S for calcium and Sirius red dye for collagen detection and quantification. RANKL expression was assessed utilizing an ELISA kit. To assess adipocyte differentiation, lipid droplets (LD) were stained with Bodipy 493/503. Mitochondria were stained with MitoTracker™ Deep Red. Both were analyzed by confocal microscopy. LD number and size were measured, and colocalization with mitochondria was determined. RT-qPCR was used to amplify genes related to adipocyte differentiation and lipid metabolism (PPARγ, CEBPα, CEBPβ, HSL, DGAT1, DGAT2, FASN, LPL, ATGL SREBP1, SREBP2, Leptin, and AdipoQ). Glycerol release, intracellular triglycerides and cholesterol were quantified with commercial kits. *Ba* infects MSCs without affecting organic and mineral matrix deposition during osteoblast differentiation, but it upregulates RANKL expression ($p<0.05$). *B*a infection modulates adipocyte differentiation by influencing lipolysis, lipogenesis, and LD- mitochondria interactions ($p<0.001$). This results in increased cellular cholesterol levels and reduced intracellular triglycerides, and glycerol release ($p<0.001$). These changes lead to larger LD. We observed increased IL-6 secretion and a higher leptin/adiponectin ratio ($p<0.01$). These effects were independent of a functional type IV secretion system, as heat-killed bacteria and bacterial DNA alone could replicate the effects of *Ba* infection. Thus, *Ba* does not inhibit the differ-

entiation of MSCs into osteoblasts. However, it promotes adipogenesis, leading to the creation of a proinflammatory profile which could contribute to bone resorption.

223. 146. **BRUCELLA ABORTUS-ACTIVATED MICROGLIA REQUIRES TYPE I INTERFERONS TO ENHANCE NITRIC OXIDE PRODUCTION AND INDUCE NEURONAL DEATH**

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Neurobrucellosis is a severe complication of brucellosis, an infectious disease caused by bacteria of the genus *Brucella*, which presents inflammatory and neurodegenerative signs. We have previously demonstrated that *B. abortus*-infected microglia induces neuronal death through primary phagocytosis of live neurons. This mechanism of cell death requires two conditions to take place: the release of nitric oxide (NO) by microglia and the increase in microglial phagocytic capacity. We have previously demonstrated that IL-6 trans-signaling pathway upregulates phagocytic activity of microglia, without affecting the production of NO. Therefore, IL-6 regulates only one of the two requirements necessary to induce primary phagocytosis of neurons. Since type I interferons (IFN) have been implicated in the regulation of NO secretion in different models of infectious diseases, we aimed to investigate if they are also relevant in modulate the NO release in *Brucella*-infected microglia. In first place, we determined that primary cultures of murine microglia infected with *B. abortus* increase gene expression of INF-α and IFN-β (p<0.05) by RT-qPCR. Then, we infected or not neurons/microglia co-cultures with *B. abortus* in the presence of IFNAR (type I interferon receptor) neutralizing antibody or its isotype control, and we evaluated neuronal density by fluorescence microscopy. We also measured NO production by the colorimetric Griess reaction, gene expression and protein level of iNOS by RT-qPCR and Western Blot, respectively. Neutralization of IFNAR completely inhibited neuronal loss caused by *B. abortus*-infected microglia (p<0.005). Moreover, neutralization of IFNAR completely abrogates NO secretion (p<0.005) by *B. abortus*-infected microglia as a result of the downregulation of iNOS gene expression (p<0.0005) as well as decreasing the level of iNOS protein (p<0.005). Altogether our results indicate that type I IFN are involved in the production of NO by enhancing iNOS synthesis, which is crucial to induce phagocytosis of viable neurons by *B. abortus*-infected microglia.

224. 155. **MODULATION OF LIPID METABOLISM AND SARS-COV-2 REPLICATION IN HCV-INFECTED HEPATOCYTES**

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Recent studies indicate increased liver injuries in COVID-19 patients with Hepatitis C virus (HCV) infection. Hepatocytes, expressing ACE2, suggest potential HCV and SARS-CoV-2 coinfection. Both viruses exploit lipid metabolism, prompting an evaluation of their combined impact on hepatocytes. Huh7.5 cells were exposed to HCV at a multiplicity of infection (MOI)=1. After 3 days post-infection (dpi), the cells were exposed to SARS-CoV-2 variants (Wuhan and Omicron BA.5) at an MOI of 0.01. Infectivity and replication were assessed at 1, 2, 3, and 6 dpi using specific RT-qPCR for HCV and RT-qPCR along with anti-N flow cytometry for intracellular SARS-CoV-2. Critical receptors for the entry and spread of both viruses in these cells, such as tetraspanins (CD9 and CD81), ACE2, and transferrin receptor (CD71), were analyzed using flow cytometry. Lipid droplets were stained using Bodipy 493/503 and analyzed using confocal microscopy. Huh7.5 cells infected with HCV were more susceptible to SARS-CoV-2 variants compared to uninfected cells (p<0.01). Increased replication of SARS-CoV-2 was associated with higher expression of ACE2, transferrin receptor (CD71), and tetraspanin (CD9) in HCV-infected cells compared to uninfected controls (p<0.001). No differences were found in expression of CD81 compared to the control of uninfected cells. Given that tetraspanins are involved in HCV entry, further investigation showed that HCV replication significantly increased by day 6 in cells coinfected with SARS-CoV-2 (p<0.01). Additionally, HCV

was found to induce the accumulation of lipid droplets in hepatocytes, a process exacerbated by HCV/SARS coinfection. The accumulation of lipid droplets was also observed in the presence of UV-inactivated HCV, suggesting that a structural component could be responsible. Further studies are necessary to establish a link between alterations in lipid metabolism, enhanced replication of SARS-CoV-2 and the increased liver damage observed in COVID-19 patients coinfected with HCV.

225. 161. **SALMONELLA TYPHIMURIUM INDUCES IMMUNE PARALYSIS IN HUMAN PERIPHERAL MONOCYTES**

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LPS tolerance, a well-described phenomenon, is characterized by immune paralysis. Recent studies suggest that this paralysis occurs due to metabolic reprogramming and epigenetic changes in immune cells after an initial stimulus, leading to anergy and an inability to respond to subsequent stimuli. This process is a form of innate immune memory. This study aimed to investigate whether *Salmonella* Typhimurium can induce changes in innate immune cells, specifically exploring its capacity to elicit either trained immunity or tolerance. Using an *in vivo* protocol for trained immunity, human adherent monocytes were isolated from multiple donors and stimulated with live and heat-killed strains of *Salmonella* Typhimurium. Following a wash and a six-day resting period, the monocytes were re-stimulated with LPS and Pam3Cys.Pro-inflammatory cytokines were measured from supernatants at various time points. Additionally, lactate production, a key metabolite in these processes, was assessed. The findings indicate that *Salmonella* Typhimurium induces tolerance in human adherent monocytes, as evidenced by a lack of response to the second stimuli. This non-responsiveness is physiologically significant, as it may prevent tissue damage during sepsis by curbing excessive inflammatory signals. These findings may have significant implications for the management of sepsis and other conditions characterized by dysregulated immune responses.

226. 166. **IMMUNOMODULATORY, ANTIOXIDANT AND ANTIVIRAL EFFECTS OF HUMAN AMNIOTIC MEMBRANE AGAINST VIRUSES CAUSING IMMUNOPATHOLOGIES.**

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Background: Viruses, such as Herpes Simplex type 1 virus (HSV-1) and Human Respiratory Syncytial Virus (RSV), cause injury by direct cellular damage or through the immunoinflammatory response, inducing the release of cytokines and chemokines involved in the immunopathogenesis of diseases. Human Amniotic Membrane (hAM) derivatives have shown antiinflammatory, antioxidant, immunomodulatory, antimicrobial and regenerative properties, showing promising properties for treating viral pathologies. Objective: To evaluate the immunomodulatory, antiinflammatory, antioxidant, and antiviral activities of hAM extracts against HSV-1 and RSV infection *in vitro*, in ocular, pulmonary and inflammatory cell lines. Methods: hAM extracts cytotoxicity was evaluated using the MTT colorimetric method after 24 h of treatment in the epithelial cells HCLE, IOBA-NHC, A549 and Vero, and THP-1 macrophages. hAM immunomodulatory activity was evaluated by ELISA kits for cytokine detection in supernatants of cell cultures exposed to viruses and hAM at nontoxic concentrations. hAM antioxidant activity was measured with fluorescent probe DCDCDHF in infected cells. The antiviral activity of hAM was determined in infected eye and lung epithelial cells by viral yield reduction assays. Statistical analysis was performed with ANOVA followed by Tukey tests (n>2). Results: No cytotoxicity was observed at the different concentrations of hAM tested. hAM modulated cytokine secretion in the different cell types and significantly decreased the production of reactive oxygen species. A significant decrease in

viral replication was observed after the addition of hAM, in eye and lung epithelial cell lines. Conclusion: hAM exerts antiviral, immunomodulatory and antioxidant effects which make it a promising candidate for the treatment of immunopathologies triggered by viruses like HSV-1 and RSV. Future assays will help elucidate the molecular mechanisms responsible for hAM healing properties.

227. 171. POLYMORPHISMS OF CCR5, IL-6, IFN-GAMMA AND IL-10 GENES IN CUBAN HIV/AIDS PATIENTS

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Introduction: Genetic studies have shown associations of several single nucleotide polymorp-hisms (SNP) with different rates of progression and variation in susceptibility to HIV infection.This study aimed to estimate the frequency of ccr5 _32, IL-6-174G/C, IFN- _+874T/A and IL-10-1082A/G polymorphisms in Cuban HIV-infected patients and a group of sero-discordant couplesto assess their influence on risk and disease progression. *Methods:* A cross-sectional study was carried out on 120 subjects registered at the Instituteof Tropical Medicine «Pedro Kour» (IPK) and the Ameijeiras Hospital from June 2018 untilDecember 2019. The amplification of fragments of the *ccr5*, *IL-6*, *IFN- _* and *IL-10* genes wasperformed by polymerase chain reaction followed by identification of polymorphisms using therestriction fragment length polymorphism analysis for IL-6 with the restriction enzymes Nla III.Amplification refractory mutation system was used for *IFN- _* and *IL-10* genes.*Results:* The allelic and genotypic distributions of the genes *ccr5*, *IL-6*, *IFN- _* and *IL-10* did notdiffer significantly between the two groups. Cell counts and plasma viral load values did notdiffer significantly between genotypes of the *ccr5*, *IL-6*, *IFN- _* and *IL-10* genes. Only the IL-6GC genotype was associated with higher viral load values. The combination of alleles of thefour considered SNPs showed a highly significant increase in the risk of HIV infection for one ofthem, but with a very low frequency (< 1%).*Conclusion:* This study contributes to evaluating the frequency of these polymorphisms andtheir influence on biomarkers of the progression of HIV infection in the Cuban HIV-population.

228. 174. MAST CELLS PLAY AN IMPORTANT ROLE IN ACTINOMYCETOMA DEVELOPMENT IN MICE

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Background: Mast cells are involved in immune responses against infections, but their role in developing actinomycetoma caused by *Nocardia brasiliensis* is unknown. **Objectives:** Evaluate the role of mast cells in the establishment of actinomycetoma by *Nocardia brasiliensis* in an experimental model of C57BL/6 mice. **Methods:** Two groups of C57Bl/6 were used, one wild-type (WT) C57BL/6 mice and a mast cell-deficient Kit mutant group (W-sh) C57BL/6-KitW-sh/W-sh mice. Both were infected with a bacterial strain of *Nocardia brasiliensis* (ATCC 700358) in the foot pad of the left hind limb. We aimed to compare the development of actinomycetoma in both groups to evaluate the role of mast cells in establishing the disease. The results were statistically analyzed using an unpaired T test. **Results:** We found that the clinical evolution of mycetoma showed a higher level of inflammation in the (W-sh) mice compared to the (WT) mice. On day 45, the inflammation level was SD= (*P<0.05), while on day 52, it was even more significant with SD= **P<0.005. The increase in inflammation levels during the chronic stage in the case of (WT) resulted in the control of the infection with $1x10^4$ colony-forming units (CFU); however, for the (W-sh) mice, a failure to control the infection was observed, with more than $4x10^6$ CFU. **Conclusion:** Mast cells are important to clear infection in this experimental model of actinomycetoma. Although (W-sh) mice exhibit inflammation in the chronic stage of actinomycetoma, they don´t achieve the same level of infection control as (WT) mice. This is likely due to the absence of mast cells in this deficient model, which leads to ineffective local inflammation that fails to eliminate pathogens. Therefore, it´s important to re-evaluate the significance of these cells in the innate immune system, even in intracellular infections.

229. 209. IMMUNOMODULATORY EFFECT OF RIFAMPICIN ON HUMAN MACROPHAGES STIMULATED WITH Mycobacterium tuberculosis TREATED OR NOT WITH ADRENAL STEROIDS

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Tuberculosis (TB) is a major health problem worldwide. The etiologic agent, *Mycobacterium tuberculosis* (Mtb) is transmitted by air and captured by lung macrophages (Mf). Mf activation along with an efficient cellular immune response (IR) are required for Mtb elimination, however these activated cells can also mediate tissue damage. We early demonstrated that newly diagnosed TB patients showed an immune-endocrine imbalance: high plasma levels of pro- and anti-inflammatory mediators and cortisol (Gc), as well as very low Dehydroepiandrosterone (DHEA) levels. During specific anti-TB treatment, the proinflammatory mediators and DHEA levels reached values like those found in healthy controls. Rifampicin (R) is a potent antimicrobial agent and a major drug in TB treatment. There is evidence that R also modulates the host IR, influencing lymphocyte migration, cytokine production, phagocytosis; and could activate enzymes of the cytochrome P450 family. Accordingly, we analyzed the effect of R (30 and 45 µg/ml) on the proinflammatory response of Mf derived from monocytes from healthy volunteers (MDMh, n=8) stimulated with irradiated Mtb (Mtbi), added or not with DHEA (10^{-7}M) and/or cortisol (10^{-6}M). All MDMh cultures were stimulated and treated for 24 hrs. Culture supernatants of Mtbi-stimulated MDMh had increased contents of IL-1β, compared to non-stimulated Mf ($p<0.05$). Treatment with R decreased IL-1β production in stimulated MDMh cultures ($p<0.05$ vs. those only stimulated) in a dose-dependent way. Similar results were achieved in Mf+DHEA+GC+Mtbi cultures, with the lower amount of IL-1β found in cultures of Mf+DHEA+GC+Mtbi+R45. A similar behavior was observed when quantifying the inflammatory cytokine IL-6 in these conditioned media.
These results suggest that R would have the capacity to modulate the activation of a key cell in the response against Mtb, such as the Mf, an effect that would be evident even in a stressful media, like in the presence of Cortisol.

230. 213. CLINICAL AND PULMONARY FUNCTION ANALYSIS IN LONG-COVID REVEALED THAT LONG-TERM PULMONARY DYSFUNCTION IS ASSOCIATED WITH VASCULAR INFLAMMATION PATHWAYS AND METABOLIC SYNDROME

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Introduction: Long-term pulmonary dysfunction (L-TPD) is one of the most critical manifestations of long-COVID. This lung affection has been associated with disease severity during the acute phase and the presence of previous comorbidities, however, the clinical manifestations, the concomitant consequences and the molecular pathways supporting this clinical condition remain unknown. Our aim was to determine the signaling pathways associated L-TPD in patients with long-COVID by analyzing clinical parameters and functional tests supported by machine learning and serum proteome profiling. **Methods:** Patients with L-TPD were classified according to the results of their computer-tomography (CT) scan and diffusing capacity of the lungs for carbon monoxide adjusted for hemoglobin (DLCOc) tests at 4 and 12-months post-infection. **Results:** Regarding the acute phase, our data showed that L-TPD was favored in elderly patients with hypertension or insulin resistance, supported by pathways associated with vascular inflammation and chemotaxis of phagocytes, according to comput-

er proteomics. Then, at 4-months post-infection, clinical and functional tests revealed that L-TPD patients exhibited a restrictive lung condition, impaired aerobic capacity and reduced muscular strength. At this time point, high circulating levels of platelets and CXCL9, and an inhibited FCgamma-receptor-mediated-phagocytosis due to reduced FcγRIII (CD16) expression in CD14+ monocytes was observed in patients with L-TPD. Finally, 1-year post infection, patients with L-TPD worsened metabolic syndrome and augmented body mass index in comparison with other patient groups.
Discussion: Overall, our data demonstrated that CT scan and DLCOc identified patients with L-TPD after COVID-19. This condition was associated with vascular inflammation and impair phagocytosis of virus-antibody immune complexes by reduced FcγRIII expression. In addition, we conclude that COVID-19 survivors required a personalized follow-up and adequate intervention to reduce long-term sequelae and the appearance of further metabolic diseases

231. 221. **IMMUNOMODULATORY EFFECT OF 7-OD ON CD4 T CELL METABOLISM IN THE CONTEXT OF HIV-TB COINFECTION**

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is exacerbated by HIV, leading to accelerated disease progression. Our group identified the hormone 7-oxo- dehydroepiandrosterone (7-OD) as a potent inducer of Th1 immune response HIV-TB co- infection context. Given that cellular metabolism determines the proper activation and response of immune cells to infection, we investigate whether the observed effects of 7-OD are associated with metabolic pathways.
We assessed the effect of 7-OD on CD4+ T-cell glucose metabolism using PBMC from healthy donors and HIV-TB patients. Monocyte-derived macrophages were infected with virulent *Mtb* (H37Rv) and co-cultured with autologous lymphocytes treated with 7-OD. Additionally, PBMCs from HIV-TB coinfected patients were stimulated with inactivated *Mtb* (*iMtb*) and 7-OD. We evaluated T lymphocyte subpopulations and glucose uptake using flow cytometry; glucose and lactate concentrations in supernatants by colorimetric assays, and IFN- γ by ELISA. In co-culture assays, CD4+ T-cell glucose uptake increased upon interaction with *Mtb*- infected macrophages. Furthermore, the treatment with 7-OD led to a significant increase in glucose uptake by CD4+ T-cells and central memory, naive, and Th1 antigen-specific subsets. Also, enhanced lactate production was observed. Both HD and HIV-TB patients' PBMCs showed a significant increase in lactate production following stimulation with *iMtb* compared to unstimulated controls. In HD, neither *iMtb* stimulation nor 7-OD treatment altered glucose uptake in total CD4+ T-cells. However, HIV-TB patients presented a significant increase in glucose consumption and uptake in total CD4 T-cells, and naive, effector memory, terminal effector, and Th1 subsets, following *iMtb* stimulation. Remarkably, this increase was further enhanced after 7-OD treatment. In conclusion, 7-OD treatment boosts glycolytic metabolism in CD4+ T-cells, which is essential for an effective immune response to *Mtb*, particularly in HIV-TB coinfected individuals with compromised immune function.

232. 237. **THE PRESENCE OF BRUCELLA ABORTUS MODIFIES THE PHENOTYPE AND FUNCTIONS OF MONOCYTE-DERIVED MACROPHAGES**

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Brucellosis is a zoonotic disease caused by bacteria of the genus *Brucella*. *Brucella* can invade and persist within various cell types, predominantly monocytes and macrophages, affecting multiple tissues and organs. If *Brucella*-host interaction modulates monocytes differentiation into macrophages remain unclear. The aim of this study was to characterize the functions of macrophages derived from monocytes differentiated in the presence of *B. abortus.* Macrophages were obtained by differentiating peripheral blood monocytes using M-CSF in the presence or ab-

sence (control) of heat-killed *B. abortus* (HKBa) for 5 days. Then, macrophage were characterized by flow cytometry. The expression of HLA-ABC and DR, CD86 and CD54 decreased in the presence of HKBa compared to the control ($p<0.01$, $p<0.0001$, $p<0.01$ and $p<0.05$, respectively). Moreover, we analyzed the expression of M1 (CD64) and M2 markers (CD209, CD206 and CD163), finding lower level of CD64 ($p<0.05$) and higher levels of CD209 ($p<0.05$) and CD206 ($p<0.0001$), indicating a M2 polarization on macrophages differentiated in presence of HKBa. We analyzed the ability of HKBa to modulate diverse functional aspects of monocyte-derived macrophages. Macrophages obtained in the presence of HKBa secrete higher amounts of IL-6 and IL-1β compared to the control ($p<0.001$). Additionally, the phagocytic capacity was evaluated by engulfment of *Candida albicans,* observing a greater phagocytic activity when macrophages were differentiated in the presence of HKBa ($p<0.05$). Finally, macrophages differentiated in the presence of HKBa showed less ability to induce CD4+ and CD8+ T cell proliferation, which correlates with MCH I and II expression levels. In conclusion, *B. abortus* is capable of modulating the phenotype of monocyte-derived macrophages, promoting less expression of antigen presenting molecules and highly expression of M2 markers, which correlates with a less T-cell activation. On the other side, these macrophages secrete higher levels of pro-inflammatory cytokines and show an increased phagocytic activity.

233. 250. **CYTOKINE QUANTIFICATION AS A POTENTIAL BIOMARKER FOR HTLV-1-ASSOCIATED MYELOPATHY (HAM/TSP)**

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Background: HTLV-1-associated myelopathy (HAM/TSP) is a demyelinating neurodegenerative for which there is no effective treatment available. Therefore, identifying biomarkers is an important step to improve the monitoring of patients with the disease. **Objectives:** Identify potential immune biomarkers of HAM/TSP through cytokine quantification. **Methodology:** HTLV-1 patients who were being followed up at the Institute of Infectious Diseases “Emilio Ribas” were invited to participate in this study. Blood samples were collected from asymptomatic carriers (AC) (n=13), individuals with the intermediate syndrome (IS) (n=16), HAM/TSP patients (n=16) and healthy controls (HC) (n=8). Then, peripheral blood mononuclear cells (PBMCs) were separated to perform the CBA quantification of IFNα, IL-12p70, MIP-1α, MIP-1β, RANTES, TNF, IL-2, IL-6, IL-4 e IL-10 with the cells supernatant, and the spontaneous production of IFNγ through ELISPOT.
Results: We observed an increase in IL-2 levels in the IS (p=0,01) and HAM (0,007) groups compared to healthy controls. Besides that, the pro-inflammatory cytokines IL-6 (p=0,01) and TNF (p=0,02) were elevated in the IS group compared with the HC group. In the spontaneous production of IFNγ, a progressive increase was noted. **Conclusion:** Alterations in cytokine profiles were observed in HTLV-1-infected individuals at different stages of infection. Among the cytokines analyzed, the progressive increase of IFNγ production seems to be the most promising biomarker.

234. 255. **EFFECTS OF IL-33 TREATMENT ON THE PROGRESSION OF TRYPANOSOMA CRUZI AND THE IMMUNE RESPONSE IN EARLY STAGES OF INFECTION**

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IL-33 is a cytokine from the IL-1 family constitutively expressed in the nuclei of epithelial and stromal cells. Upon cellular damage or tissue injury, it is released into the extracellular space as an “alarmin” that activates the immune system. IL-33 signals through its receptor, the ST2 molecule, and promotes the activation and differentiation of various immune cell populations. The biological roles of IL-33 during infections, both beneficial and deleterious, have been shown to result from not completely elucidated and context-dependent mechanisms. Our objective is to determine the impact of the IL-33/ST2 pathway on the immune mechanisms that promote inflammation, microbial control, and tissue repair during the acute phase of *Trypanosoma cruzi* infection and how

this affects progression to the chronic stage. To this end, C57BL/6 Foxp3-GFP mice were infected with 5000 *T. cruzi* tripomastigotes (Tulahuén strain), treated at 0, 3 and 6 days post-infection (dpi) with recombinant IL-33 or PBS (controls). Notably, IL-33-treated mice exhibited a peripheral lymphocytosis compared to PBS (p=0.0001) at the peak of parasitemia, around 21 dpi. Flow cytometry evaluation of immune infiltrates in the spleen, liver, and skeletal muscle at different dpi showed that IL-33 administration increases the number of tissue-repair regulatory T cells (rtTreg) (p=0.0059), as well as of other leukocyte populations such as type 2 innate lymphoid cells (ILC2) and *T. cruzi* specific CD8+ T cells with different kinetics. Additionally, IL-33 treatment favorably impacted the progression of acute *T. cruzi* infection by reducing tissue damage and weight loss, promoting parasite control and improving survival (80% vs. 42% with PBS, p=0.0043). Further studies are needed to dissect the relevance of different immune subsets in the protective effect of IL-33 treatment during the acute phase of *T. cruzi* infection and to determine the impact of the IL-33/ST2 pathway in progression to the chronic stage.

235. 266. **THE DUAL ROLE OF FOLLICULAR CYTOTOXIC CD8+ T CELLS IN TRYPANOSOMA CRUZI INFECTION: PROMOTING B CELL DIFFERENTIATION INTO ANTIBODY-SECRETING CELLS WHILE MAINTAINING CYTOLYTIC PROPERTIES**

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Experimental infection with *Trypanosoma cruzi* triggers an early plasmablast response in the spleen of C57BL/6 mice during the acute phase. This response coexists with a subset of $CD8^+$T cells known as follicular cytotoxic T cells (Tfc, $CD8^+CXCR5^+PD\text{-}1^+$), which peaks at 18 days post-infection(dpi) and begin to decline at 23 dpi. Tfc expressed markers commonly associated with Tfh cells, including ICOS, CD40L, and Bcl6. We aimed to assess the role of Tfc cells considering their potential helper functions and inherent cytotoxic capabilities. To achieve this, we characterized Tfc and Non-Tfc cells ($CD8^+CXCR5^{neg}PD\text{-}1^{neg}$) using bulk RNA sequencing. GSEA analysis revealed an enrichment of pathways associated with B cell activation in Tfc cells. We further evaluated the impact of Tfc cells on B cell responses through *in vitro* assays, co- culturing Tfc or Non-Tfc cells with B cells. Sorted Tfc and Non-Tfc cells from infected mice were co-cultured with B cells stimulated with CpG plus anti-CD40, or with B cell stimulated with anti-IgM plus anti-CD40; or with naïve B cells from infected or non- infected mice at 2:1 ratio (2CD8+:1B-cell) for 20 hours. Flow cytometry revealed a higher frequency of $B220^+CD138^+Blimp\text{-}1^+$ and $B220^+IgD^{neg}$ cells in naïve or activated B-Tfc co-cultures compared to B-Non-Tfc and B cells alone ($p<0.05$). Additionally, multiplex bead-based assay showed significantly higher levels of different immunoglobulin isotypes in the supernatants of B-Tfc co-cultures, indicating that Tfc promote B cell differentiation into antibody-secreting cells (ASC). Trans-well experiments indicated that cell-cell contact was essential for the differentiation of naïve B cells into ASC. Interestingly, co-culturing plasmablasts with either Tfc or Non-Tfc cells led to a significant reduction in viable plasmablasts after 20 hours. Blocking Fas/Fas-L interaction reversed plasmablast death. Our findings demonstrated that Tfc cells promoted B cell differentiation into ASC through an antigen-independent mechanism while also inducing cytotoxicity on plasmablasts via Fas/Fas-L interaction.

236. 267. **CARBAPENEMASE-PRODUCING K. PNEUMONIAE ST258 EVADES NEUTROPHIL KILLING EVEN IN THE PRESENCE OF NATURAL ANTIBODIES**

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Carbapenemase-producing *Klebsiella pneumoniae* from the sequence type 258 (Kp) are associated with significant mortality in immunocompromised patients. Previously, we have demonstrated that Kp does not induce neutrophil

extracellular trap (NETs) formation and can inhibit PMA-induced NETs. Natural antibodies are key immune components of blood enhancing the immune response against pathogens. Therefore, we asked whether natural antibodies in human serum could enhance the neutrophil response against Kp. To evaluate the effect of natural antibodies, a pool of human serum (HS) was obtained from peripheral blood of healthy donors and complement was heat inactivated. Kp was pre-incubate for 30 minutes with HS (Kp-HS) and Kp-HS was used as a stimulus. The level of opsonization of Kp-HS was first assessed using flow cytometry with FITC-conjugated anti-human IgG or IgM antibodies. We observed that Kp-HS was opsonized by 17% IgM and 5% IgG antibodies. Next, we investigated the effect on neutrophil-microbicidal functions, such as phagocytosis, ROS production, NETs formation, and killing. We used GFP-expressing Kp to determine the percentage of phagocytosis by flow cytometry. The percentage of GFP^+-neutrophils showed a significant increase in Kp-HS phagocytosis compared to Kp ($p<0,05$). Moreover, ROS production, determined by flow cytometry with DHR, significantly increased with Kp-HS ($p<0,05$). However, Kp-HS could not induce NETs formation and still inhibited PMA-induced NETs formation, as determined by DNA released levels and MPO activity, two key NETs components. To address if HS could modulate Kp elimination by PMN, we assessed intracellular killing after 1 hour of incubation. However, there were no significant differences in colony forming unit (CFU) counts between Kp and Kp-HS. Our results indicate that natural antibodies in human serum cannot reverse Kp's evasion from neutrophil-mediated death.

237. 278. **MANNOSYLATED ISONIAZID POLYMERIC MICELLES FOR AN OPTIMIZED TREATMENT OF LATENT TUBERCULOSIS IN AN IN VITRO GRANULOMA MODEL**

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Background. It is estimated that one quarter of the global population has latent tuberculosis infection (LTBI). Isoniazid (INH) is the main anti-tuberculosis drug for LTBI treatment. Given that this drug has serious side effects, including liver failure and hepatotoxicity, the development of new inhalable delivery system formulations is urgently needed. **Objectives.** Investigate the anti- TB effectiveness of INH in polymeric micelles (INH-MP) with or without mannose-ligand (INH-(0.1%man)MP and INH-(0.5%man)MP) for dormant *Mycobacterium tuberculosis* (*Mtb*) in an *in vitro* granuloma model. **Methods & Results.** First, we analyzed the microbicidal effect of INH-MP and INH-(man)MP (0.005, 0.01 & 0.05 µg/ml) on active and dormant *Mtb*H37Rv cultures. We observed a reduced colony forming units (UFC/ml) of the INH-(0.1%man)MP and INH-(0.5%man)MP compared with the soluble antibiotic INH (0.01 µg/ml) after 7 days of treatment (ANOVA $p<0.05$). Then, we established and characterized a 3D *in vitro* LTBI granuloma model. In this cellular context, the expression of CD19 was not altered, and the co-expression of CD3 and CD4 was slightly increased, determined by flow cytometry. Additionally, the increase of CD14 was observed and the expression of mannose receptor CD206 remained unchanged. Finally, we found a decrease of UFC/ml of dormant intracellular *Mtb*H37Rv after 7 days of treatment with INH-MP, INH-(0.1%man)MP and INH-(0.5%man)MP respect to INH soluble 0.01 µg/ml (ANOVA $p<0.05$). **Conclusion.** INH-(man)MP showed promising results as an optimized treatment of latent TB.

238. 283. **ANTIMICROBIAL AND PROTECTIVE EFFECT OF ALOE VERA GEL IN CLOSTRIDIOIDES DIFFICILE INFECTION.**

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Clostridioides difficile is a Gram-positive bacil-

lus, anaerobic, spore-forming that is the main cause of hospital-acquired diarrhea. *C. difficile* infection (CDI) symptoms can range from mild to severe diarrhea and even death. CDI treatment, in Argentina, is based on administration of metronidazole or vancomycin. The emergence and spread of *C. difficile* isolates resistant to multiple antibiotics is becoming an increasing problem for treatment of CDI. Aloe Vera (AV) has been used in traditional herbal medicine as an immunomodulatory agent with antimicrobial properties. The inner leaf gel contains active compounds with prebiotic activity that inhibit the growth of pathogenic bacteria, making it an alternative therapy for intestinal dysbiosis. We evaluated the effect of *Aloe saponaria* on the growth of *C. difficile* and its impact in combination with vancomycin and metronidazole. The antibacterial activity of AV was determined by broth microdilution assays (final concentrations of 1, 5, 10 and 20%) using the hypervirulent and toxigenic *C. difficile* 027/BI/NAP1 strain. AV gel inhibits *C. difficile* growth ($p<0.01$) and significantly increases the vancomycin and the metronidazole effects ($p<0.01$). We also evaluated the effect of AV as a protector agent of the intestinal epithelia using the Caco2 cell line. Differentiated cells were treated for 24h with *C. difficile* and/or TcdA/TcdB toxins in the presence of AV. By optical and fluorescence microscopy, we observed that AV has a protective effect on the integrity of the monolayer ($p<0.01$) and increases the expression of Claudin-1 ($p<0.01$). In addition, we observed an increase in monolayer mucus production without alteration in cell viability (assessed by flow cytometry). This evidence shows a beneficial effect of AV both as an antimicrobial, reducing the concentrations of antibiotics administered, as well as a protective agent for the integrity of the intestinal epithelium, suggesting that AV could be used as a combined therapy for CDI treatment.

239. 290. **REPLENISHMENT OF LPM ENHANCE IMMUNE RESPONSE AGAINST T. CRUZI INFECTION**

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Peritoneal cavity is a space that contains fluid with immune cells concentrated 20 times more than blood. Resident peritoneal macrophages (PEMs) consist of two subpopulations with distinct origin, sizes and surface markers. Large Peritoneal Macrophages (LPM) origin in embryonic yolk sac, are CD11b+F4/80+MHC-IIneg and can self-replenish. Small Peritoneal Macrophages are CD11b+MHC-II+F4/80med and derive from blood monocytes. Under homeostasis LPM represents more than 70 percent of total CD11b+ cells, but during infection or inflammation dampens drastically in a process known as Macrophage Disappearance Reaction. LPMs are essential for T cell priming in *T. gondii* infection and its pleural analogous control lung *L. sigmodontis* infection. In *T. cruzi* infection, we observed a reduction of LPMs during acute phase in susceptible Balb/c mice that did not restore in chronic phase. Instead, this population was replaced by 'Converting macrophages', which do not acquire LPM maturation characteristics. We hypothesized that restoring LPM in the peritoneal cavity could control parasite replication and enhance T cell response. To investigate how the replenishment of LPMs impacts the response to *T. cruzi* infection, we transferred PEMs to infected mice early in the acute phase by i.p. injection. Four days later, blood samples, peritoneal lavages and spleens were collected. Infected animals without PEMs transfer and non-infected animals were analyzed in parallel. We found that mice transferred with PEMs showed less parasitemia than non-transferred infected animals ($p<0.05$). Both spleen and peritoneal T cells showed the same CD4 activation but CD8 T cells exhibited higher PD-1 positive cells than non-transferred counterparts ($p<0.05$). Spleen CD8 T cells showed more frequencies of effector (CD44+CD62-Lneg) cells ($p<0.01$). These findings suggest that LPM restoration may be a promising strategy to enhance immune response and control parasitic replication during *T. cruzi* infection.

240. 291. **METFORMIN PREVENTS META-INFLAMMATION IN T. CRUZI INFECTION**

Ruth Eliana Baigorri[1], Yanina L. Mazzocco[1], Ma. Florencia Hellriegel[1], Camila Fontanari[1], Matías E. Vázquez[1], Pilar Aoki[1], Cinthia C. Stempin[1], Fabio M. Cerbán[1]
1. CIBICI-CONICET

Metformin, an anti-diabetes drug, can modulate inflammation-related pathways. Metformin treatment reduces inflammatory cytokines levels, ROS production and neutrophil activation. *T. cruzi* infection is associated with cardiac and hepatic injury due to parasite nests, cell infiltrate and re-

active metabolites production. We previously discovered that *in vivo* treatment of infected Balb/c mice with Metformin resulted in lower parasitemia compared with non-treated counterparts. Additionally, peritoneal macrophages showed a decreased iNOS expression and ROS production. We hypothesize that Metformin treatment could reduce inflammation and tissue damage of parasite-affected organs. We treated infected animals with Metformin via oral gavage from 5 to 18 days post-infection (d.p.i.) and then blood samples, spleens and hearts were collected. We found that Metformin treatment decreased total spleen cell counts ($p<0.05$), plasma levels of TNF-alpha ($p<0.05$), IL-6 ($p<0.01$), but IL-10 remained unchanged in both groups. Heart tissue analysis revealed less cell infiltration in Metformin-treated animals compared to untreated and infected groups. Serum biochemical analysis exhibits reduced muscle damage, with lower CPK ($p<0.05$) and LDH levels. To investigate how Metformin could prevent cardiac damage by reducing infiltrate, we examined hearts of animals at 100 d.p.i. that were treated during the acute phase. Collagen fibers assessed by Picrosirius Red stain under polarized light were less abundant in Metformin-treated than untreated mice ($p<0.05$). However, survival analysis of both groups of infected animals showed no significant differences. This could be partly due to potential liver damage, as infected mice exhibited elevated GOT and GPT ($p<0.05$) plasma levels during acute phase, with higher levels in treated animals. These findings suggest that while Metformin reduces inflammation and heart tissue damage during *T. cruzi* infection, its impact on survival may be limited by potential liver toxicity which aggravates infection damage.

241. 294. **NEUTROPHIL EXTRACELLULAR TRAPS (NETS) AND NET-ASSOCIATED PROTEINS RESTRAIN HIV-1 INFECTION IN MACROPHAGES**

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Neutrophils have key roles in innate immune responses and can release extracellular traps (NETs), which are structures composed by chromatin associated with granule and cytoplasmic proteins. These traps are released upon neutrophils' contact with numerous factors, including infectious agents that can be entrapped and inactivated by NETs. Since neutrophils are recruited to the sites of HIV-1 replication in lymphoid tissues, where they are activated by inflammatory mediators, we investigated whether these structures modify HIV-1 production by macrophages and recently published that exposure of HIV-1-infected macrophages to NETs resulted in inhibition of viral replication, through reducing HIV-1 genome integration and infectivity of residual virions. Now we report new molecular mechanisms involved in HIV-1 inhibition by NETs. Monocyte-derived macrophages, obtained by density gradient centrifugation and adherence onto plastic plates, were infected *in vitro* by HIV-1 and exposed to NETs or to NET- associated proteins. ELISA method was used to measure HIV-1 replication and inflammatory mediators in culture supernatants. Similar assays were performed lymphocytes. Treatment of macrophages with NETs before HIV-1 infection also reduced viral replication. Inhibition of Toll-like receptor adaptor protein MyD88 did not revert NET-mediated HIV-1 inhibition. We found that, likewise NETs, the NET- associated proteins neutrophil elastase, myeloperoxidase, HMGB1 and S100A8/A9 inhibited HIV-1 replication, in concentrations similar to those found in NETs, and were required for NET-mediated HIV-1 inhibition. NETs increased macrophage production of β-chemokines and similar results concerning viral inhibition and production of these anti-HIV-1 mediators were found in lymphocytes. High levels of LPS were detected in the plasma of HIV-1 patients, positively correlating with the levels of circulating DNA-elastase complexes, suggesting that HIV-1 infection and microbial translocation contribute to NET formation. Our results indicate that NETs and their proteins function as an innate mechanism able to control the HIV-1 infection in macrophages and lymphocytes.

242. 295. **ANALYSIS OF LEUKOCYTES DYNAMICS AND EXPRESSION OF PD-1 AND PD-L1 MOLECULES IN A MOUSE MODEL OF ACTINOMYCETOMA INDUCED BY NOCARDIA BRASILIENSIS.**

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Background: Actinomycetoma is a chronic infection caused by actinomycetes such as *Nocardia brasiliensis*. As in chronic viral infections and cancer, co-inhibitory molecules, PD- 1 and PD-L1, negatively regulate the lymphocyte effector response on persistent antigenic stimulation. The role of these regulatory mechanisms in actinomycete chronic infections is currently unknown. **Objective:** To evaluate the expression of PD-1 and PD-L1 molecules on leukocytes from infected tissue and secondary lymphoid organs in a mouse model of actinomycetoma by *N. brasiliensis.* **Methods:** Experimental actinomycetoma was induced by inoculating the footpad of BALB/c mice with a suspension of *N. brasiliensis* ATCC HUJEG-1 700358. At 70-, 100- and 365- days post-infection (DPI) mice were sacrificed, tissues were collected for cytometric analysis; healthy controls were also included (n=6). Following tissue digestion, a cell suspension from each tissue was stained with an antibody cocktail for myeloid (CD45, Ly6G, F4/80, PD-L1) and lymphoid markers (CD3, CD4, CD8, PD-1). Results were expressed as percentages for cell phenotype and fold-change expression for PD-1 and PD-L1 molecules. After reviewing normality, statistical analysis was performed accordingly. **Results:** Late stages of experimental actinomycetoma showed 93.86% neutrophils and only a small proportion of lymphocytes, predominantly CD4+. The expression of PD-1 in lymphocytes increased at 70 DPI in CD4+ cells and at 100 DPI in CD8+ cells. In contrast, the ligand PD-L1 was higher in the mycetoma compared to other organs at 100 DPI. **Conclusion:** Late stages of experimental actinomycetoma have an increased proportion of infiltrating neutrophils with only a small proportion of lymphocytes, and the expression of PD-1 and PD-L1 increases with infection. This suggests that an immune exhaustion program may be present in the actinomycetoma functioning as a regulatory mechanism to control inflammation.

243. 303. **STUDY ON THE ROLE OF NUCLEAR RECEPTORS NR4A IN MACROPHAGE RESPONSE TO MYCOBACTERIUM TUBERCULOSIS STIMULATION**

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Nuclear receptors, a superfamily of ligand-dependent transcription factors, are indispensable in the immune-endocrine regulation of homeostasis and pathophysiology. NR4As receptors have emerged as important regulators of cell polarisation in immune response (IR) and inflammation, particularly affecting NF-κB signalling in macrophages (Mf). On another hand, tuberculosis is a major infectious disease caused by *Mycobacterium tuberculosis* (MTB) which infects alveolar Mf, promoting a cellular IR, becoming harmful in the long term, and being central to the immunopathology of TB. This work evaluates NR4A's participation in the Mf response exposed to MTB antigens. Regarding this, THP-1 cell line differentiated into inflammatory (M1Mf) and anti-inflammatory (M2Mf) Mf was treated with irradiated MTB (MTBi) at different times (1, 3, 6 and 24 H). We determined RNAm expression of CD80, NR4A1-3, NFKB1, NFKBIA/B, IL1β, IL6 and IL10 by RT-PCR, and IL-6 and IL-10 production in culture supernatants by ELISA. MTBi-stimulation of M1Mf significantly increased IL1β and NFKBIA RNAm compared to unstimulated ones. M2Mf stimulated with MTBi showed an augmented expression of NR4A1-3, NFKB1, NFKBIA, IL1β and CD80. Comparing unstimulated M1Mf and M2Mf, the former presented higher levels of NR4A3, NFKB1, NFKBIA, IL1β and CD80 RNAm, meanwhile MTBi treatment sustained the differences in NR4A3, NFKB1 and CD80. The data of IL-6 and IL-10 production showed significantly higher concentrations of IL-6 in M1Mf compared to M2Mf, which were increased with MTBi treatment. IL-10 concentration was higher in M1Mf than M2Mf at 1 and 3 H, although M2Mf reached higher values at 6 and 24 H. Finally, we observed positive associations between NR4A3, NFKB1, NFKBIA and IL-10 in M1Mf treated with MTBi. The coexistence of inflammatory and anti-inflammatory mediators, with an increase in NFκB inhibitors in MTBi-treated cells, may imply an attempt to regulate the inflammatory response elicited by the pathogen, wherein NR4As seem to play a regulatory role.

244. 313. **PURINERGIC SIGNALING SUBVERTS CYTOTOXIC CD4 T-CELL IMMUNE RESPONSES DURING TRYPANOSOMA CRUZI INFECTION**

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Trypanosoma (T.) cruzi infection is endemic in Latin America and is characterized by excessive regulation of immune effector mechanisms that allow parasite persistence. Hypoxia within infected tissues triggers the activation of the transcription factor HIF-1 and be linked to ATP release. Extracellular ATP exhibits microbiocidal effects but is metabolized by CD39 and CD73 ectoenzymes to adenosine (ADO), a potent immunosuppressor. We aimed to elucidate how HIF- 1 regulates ADO production by CD39 and CD73 and its impact on cytotoxic CD4 T-cell response in the setting of acute *T. cruzi* experimental infection. We found that TCR stimulation of purified CD4 T-cells induces a significant increase in HIF-1α and CD39CD73 expression. The inhibition of ATP-receptor P2X7 and the stimulation with ADO abrogated the expression of cytotoxic effector molecules (granzyme B or IFN-γ) in TCR-stimulated CD4 T-cells compared with controls maintained in medium. Besides, HIF-1 stabilization with DFO treatment reduced the rate of cytotoxic effector molecules and increased CD73 expression. Strikingly, the HIF-1α-mediated immunosuppression was reversed by CD73- inhibitor APCP treatment (DFO vs DFO+APCP; $p<0.05$). In parallel, *T. cruzi* infection increased HIF-1 expression and expanded $CD39^{+}CD73^{+}$ CD4 T-cells at 14 days post-infection (dpi), in contrast to $CD39^{-}CD73^{+}$ phenotype observed in non-infected spleens. After this time point, concomitant with a significant drop in IL-6 (0.94 vs 0.074pg/mL; $p<0.05$) and sustained IFN-γ levels (848 vs 575pg/mL; $p=0.195$), CD73 expression abruptly diminished, and remain low throughout the infection (98 vs 33%; $p<0.05$). In accord with these findings, *in vitro* neutralization of IFN-γ significantly increased CD73 expression in activated CD4 T-cells, an effect boosted by the addition of bioactive recombinant IL-6. Conversely, blocking IL-6 led to a significant decrease in CD73 levels. Summing up the results highlight how HIF-1/CD39/CD73/ADO axe subverts CD4 T-cell responses in the setting of *T. cruzi* infection and identify factors involved in driving CD73 expression.

245. 319. BACTERIAL LYSATE OM-85 REDUCES SUSCEPTIBILITY TO *P. AERUGINOSA* IN CYSTIC FIBROSIS BUT INCREASES TISSUE DAMAGE DURING THE INFECTION IN A PRECLINICAL MODEL

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Pseudomonas aeruginosa is a gram-negative bacterium frequently associated with severe infections in patients with cystic fibrosis (CF). This bacterium colonizes the lungs, creating a favorable environment for chronic infections that are difficult to treat. Over time, these infections lead to the progressive deterioration of lung function, significantly contributing to morbidity and mortality in these patients. This study investigated the efficacy of the bacterial lysate OM-85 (Broncho Vaxom®) in a preclinical CF model, aiming to evaluate its impact during *P. aeruginosa* infection. C57BL/6 mice were induced into a preclinical CF model, and they were prophylactically treated with 1 mg of OM-85 nasally and then infected with virulent *P. aeruginosa* (PA14). qPCR for bacterial DNA detection in the mice lungs demonstrated microbiota modulation by OM-85 decreasing Bacteroidetes and Gammaporteobacterias, and an increase of Firmicutes. In CF model the drug was also able to modulate the microbiome mainly increasing Bacteroidetes and Firmicutes phyla. When the mice were infected, a lower susceptibility to PA14 infection was observed, as evidenced by CFU results. Furthermore, the OM-85-treated animals showed higher production of IL-17 and IFN-γ in the lungs and decreased IL-10 compared to CF non-treated animals Additionally, there was increased influx of CD11+CD86+, CD4+IFN-γ+, and CD4+IL-17+ cells in the lung homogenate, as assessed by flow cytometry. Consistent with the cytokine levels and immunophenotyping results, histopathology revealed septal thickening, alveolar collapse, and pronounced leukocytic infiltration compared to PBS, OM-85, and CF groups. While the hepatic microstructure appeared normal in all non-infected groups, PA14, CF+PA14, and CF+OM-85+PA14 groups showed mice hepatic parenchyma expansion, collapse of sinusoidal capillaries, and increased interstitial

cellularity. These findings suggest that, despite the benefits in combating infection in CF, OM-85 prophylaxis also increases histopathological changes, warranting further studies to assess its long-term effects.

246. 323. **IMPACT OF PRIOR SARS-COV-2 INFECTION ON THE MTB-SPECIFIC ADAPTIVE IMMUNE RESPONSE.**

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Innate immune cells can be trained to enhance or suppress effector functions in response to subsequent infections, a process known as “Trained Immunity.” In the context of the COVID-19-tuberculosis (TB) co-pandemic, we aimed to investigate the effect of viral infection on trained immunity and its impact on TB progression. To this aim, macrophages and lymphocytes were obtained from healthy donors (buffy coat). Afterwards, macrophages were trained (abortive infection) with SARS-CoV-2, infected with *Mycobacterium tuberculosis* (*Mtb*) H37Rv strain, and co-cultured with autologous T lymphocytes. The *Mtb*-specific lymphocyte response was evaluated by measuring IFN- γ production in the culture supernatant using ELISA, and lymphocyte proliferation (CFSE dilution assay), CD69 and CD137 expression (activation and Ag-specific marker, respectively) were assessed on CD4+T cells, and CD69 was determined on Th1, Th1* and Th17 cells by flow cytometry. We observed that *Mtb* induced a significant increase in CD4+T cell proliferation and CD69 expression in untrained cells. However, prior training of macrophages with SARS-CoV-2 prevented the increase in *Mtb*-specific proliferation, CD69 and CD137 expression in CD4+T lymphocytes after co-culture, and abrogated the activation of Th1, Th1* and Th17 cells after *Mtb*-stimulation. Furthermore, when determining IFN-γ production in culture supernatants, macrophage training with SARS- CoV-2 caused a significant decrease in this cytokine’s production compared to that induced by untrained and *Mtb*-infected macrophages. Our results suggest that prior SARS-CoV-2 infection induces changes in macrophages, modulating the subsequent immune response against *Mtb*. These findings contribute to the knowledge base on anti- tuberculosis immunity and could be relevant for better disease management, especially in populations susceptible to *Mtb* infection/reactivation.

247. 325. ***BA* RNA INDUCES EARLY ACTIVATION BUT REDUCES PROLIFERATION AND PROMOTES APOPTOSIS IN LYMPHOCYTES**

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In order to survive inside the host, *Brucella abortus* (*Ba*) must trigger different strategies to evade the adaptive T cell response it elicits. We have recently demonstrated that *Ba* RNA (a PAMP related to pathogens’ viability or *vita*-PAMP) increases the expression of the early activation marker CD69 on peripheral blood mononuclear cells (PBMCs) independently of plate-bound anti-CD3 stimulation at 24 h. However, *Ba* RNA decreases the expression of the senescence marker CD28 only in anti-CD3 pre-stimulated PBMCs at 4 days. The aim of this study was to deepen this dual modulation of *Ba* RNA-mediated lymphocyte response. We first evaluated whether *Ba* RNA could reproduce this modulation in JURKAT T cell line. Cells were incubated with *Ba* RNA for 24 h and 4 days, and the expression of CD69 and CD28 was evaluated by flow cytometry. *Ba* RNA increased the expression of CD69 at 24 h ($p<0.05$) but decreased the expression of CD28 at 4 days, only in anti-CD3 pre-stimulated JURKAT T cells ($p<0.05$). Next, we evaluated the effect of *Ba* RNA on lymphocyte proliferation and apoptosis. For this, unstimulated or anti-CD3 pre-stimulated PBMCs were treated with *Ba* RNA for 24, 48, 72 and 96 h. Proliferation was measured by MTT colorimetric assay. *Ba* RNA decreased the proliferation both in unstimulated or pre-stimulated PBMCs at 48, 72 and 96 h ($p<0.05$). For apoptosis assays, cells were stained with Annexin V and Propidium Iodide (PI) and then evaluated by flow cytometry. *Ba* RNA increased the apoptosis (Annexin V+ cells) in unstimulated cells at 48, 72 and 96 h but did not affect apoptosis in pre-stimulated cells. Overall, our results show that *Ba* RNA initially activates lymphocytes, but later reduces proliferation and promotes apoptosis, which may

favor the establishment of a chronic infection.

248. 336. SARS-COV-2-INDUCED TRAINED IMMUNITY: EFFECTS ON MACROPHAGE RESPONSES TO *MYCOBACTERIUM TUBERCULOSIS*

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Innate immune cells develop long-term memory in response to different agents, known as “trained immunity”, which may result in either enhanced effector responses or immunological tolerance upon (re)challenge. The heterologous effects of SARS-CoV-2 infection or vaccination on macrophages, particularly regarding their response to *Mycobacterium tuberculosis* (*Mtb*), remain underexplored. Effective control of *Mtb* infection relies on a robust immune response, including M1 macrophages. Therefore, we aimed to investigate the phenotypic characteristics of macrophages subjected to SARS-CoV-2 training with infective or inactivated virus (iSARS- CoV-2), and their response to virulent *Mtb*. Using an *in vitro* model of trained immunity on monocyte-derived macrophages from buffy coats and subsequent infection with *Mtb* H37Rv, M1/M2 phenotype through surface markers were analyzed by flow cytometry. As a control, heterologous stimulation with LPS was performed. When compared to uninfected cells, a shift toward M1 phenotype upon *Mtb* rechallenge was observed in both, non-trained and iSARS-CoV-2 trained macrophages, characterized by a statistically significant increased frequency of HLA-DR, while concurrently exhibiting a decrease in CD64, a marker associated with inflammation. Nevertheless, macrophages trained with infective SARS-CoV-2 and then challenged with live *Mtb* showed the lowest proportion and molecule density (MFI) of HLA-DR and an increment in CD64+ cells with statistical significance compared to uninfected cells. This led to a statistically significant decreased ratio of HLA-DR+/M2-macrophages (CD163+ or CD209+), compared to non- trained and iSARS-CoV-2 trained groups in response to *Mtb* infection. So far, data suggest that SARS-CoV-2 trained macrophages might have impaired antigen presentation capacity, thereby adversely affecting the subsequent activation of an effective immune response against *Mtb*. A functional in-depth analysis of the effect of SARS-CoV-2 training in macrophages and pathogen-specific responses is still needed.

249. 338. HIV INFECTION OF MACROPHAGES VIA HETEROTYPIC CELL-TO-CELL CONTACT IMPAIRS THEIR DIFFERENTIATION INTO OSTEOCLASTS

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Background: Macrophages, which can serve as osteoclast precursors, show diverse responses to cytokine stimuli. While weakly infected by cell-free HIV, they are more efficiently infected via cell-to-cell transmission, delivering a higher viral load. This study aimed to investigate how cell-to-cell HIV infection affects macrophages and their differentiation into osteoclasts. **Methods**: The CD4+ T-cell line Jurkat was infected with HIV AD8-VSV using spinoculation at 0.5 pg/cell. Human primary monocyte-derived macrophages (MDM) were differentiated with M-CSF (6 days-10ng/ml), followed by RANKL (9 days-30ng/mL) to obtain osteoclasts. On day 6, cultures were exposed to HIV- infected (or non-infected) Jurkat cells at a 2:1 ratio for 18 hours. HIV infection efficiency was assessed 3 days post-co-culture by measuring intracellular p24 (flow cytometry). Osteoclasts are multinucleated, TRAP+, and actin ring formation, while Jurkat-macrophage adherence (ICAM-1/LFA-1/CD9/CD81) was differentially evaluated by flow cytometry using cell tracking. **Results**: Osteoclastogenesis was significantly ($p<0.05$) reduced when osteoclast precursors were exposed to HIV-Jurkat (Inf:19.0±8.7 vs. Ctrl:70.3±13.2), and actin ring organization was disrupted. The intracellular HIV-p24 expression raised to 24.0±6.4% among Jurkat and, after 3 days of co-culture, 36.8±18.6% of MDM were also positive, thus involving cell-to-cell transfer. When infected, the Jurkat- MDM adherence appeared significantly higher ($p<0.05$) than non-infected controls, but it decreased when CCR5 was antagonized (Inf:8.5±0.4; Inf+TAK779:5.9±0.2; Ctrl:2.8±0.7). When compared to their non-infected counterparts a significantly higher ($p<0.05$) expression of adhesion molecules (measured as MFI) was observed among HIV-infected MDMs (ICAM: $3.4\times10^5\pm1.1\times10^5$ vs. $2.8\times10^5\pm5.6\times10^4$; LFA-1: $2.9\times10^5\pm3.2\times10^4$

vs. $1.4\times10^5\pm3.4\times10^4$; CD9: $1.8\times10^5\pm4.4\times10^4$ vs. $8.8\times10^4\pm2.1\times10^4$; CD81: $4.3\times10^5\pm7.1\times10^4$ vs. $3.0\times10^5\pm4.0\times10^4$) and Jurkat cells (LFA-1: $5.1\times10^3\pm3.2\times10^2$ vs. $2.3\times10^3\pm3.0\times10^2$). **Conclusions:** Heterotypic infection of osteoclast precursors through cell-to-cell contact leads to significant HIV transfer and markedly disrupts their differentiation into osteoclasts. The LFA-1/ICAM-1 and gp120/CCR5 interactions are key drivers of this infection process.

250. 343. **REGULATORY T CELLS ACCUMULATE IN SKELETAL MUSCLE DURING CHRONIC *TRYPANOSOMA CRUZI* INFECTION AND EXHIBIT A DUAL TH1/REPAIR SPECIALIZATION**

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Regulatory T cells (Tregs) are lymphocytes with immune and non-immune modulatory properties, capable of adapting their roles through various specialization programs. *Trypanosoma cruzi* (Tc) infection serves as a model for studying different immune contexts, with an acute phase characterized by high parasite loads, inflammation, and tissue damage, followed by a chronic phase with low parasitism and persistent immune activation. During the acute phase, Tregs specialize in suppressing Th1-immunity, limiting effector responses, and delaying parasite control, but their role in chronic infection remains less understood.
Our objective is to characterize the Treg response during chronic Tc infection and correlate these findings with effector immunity, parasite load, and tissue damage. We used Foxp3-GFP-C57BL/6 mice infected with 5000 Tc parasites (Tulahuen strain) and evaluated them at 125 days post-infection. We first characterized this stage by assessing parasite load across tissues, finding that skeletal muscle (SM) harbored the highest levels. In all tissues, most CD8+ cells exhibited an effector phenotype (CD44+CD62L-), with detectable parasite-specific cells. Plasma markers of damage revealed elevated levels of LDH, GOT, and CPK-MB activities, with GPT, CPK activities, and glycemia unchanged compared to controls. Histological analysis of SM revealed necrosis and calcification of muscle fibers, along with their replacement by adipose tissue, absent in controls. Flow cytometry studies revealed a significant accumulation of Tregs (CD4+Foxp3+) in SM ($p<0.05$), a slight increase in the liver, and no significant changes in the spleen and heart compared to controls. RNA-seq analysis of SM Tregs, confirmed by flow cytometry, indicated that SM Tregs exhibit both tissue-repair and Th1-modulatory features. In conclusion, during chronic Tc infection, Tregs accumulate in SM and display characteristics of both tissue repair and Th1 modulation. Further studies are needed to elucidate their role in controlling tissue damage and the antiparasitic response observed in this tissue during the chronic phase.

251. 352. **PARTICULATE MATTER EFFECT ON THE PULMONARY IMMUNE RESPONSE AGAINST THE INFECTION WITH *PSEUDOMONAS AERUGINOSA* IN A MURINE MODEL**

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Background: Particulate Matter (PM) exposure has become a significant global public health concern. Exposure to PM has been linked to adverse health outcomes, principally in the respiratory system. Besides, several studies have shown that PM exposure impairs innate immune response mechanisms increasing the susceptibility to infectious diseases. Among these, infections caused by *Pseudomonas aeruginosa*—an opportunistic pathogen ranked as the fourth leading cause of infection-related mortality globally—have gained significant relevance for study. The impact of PM exposure on opportunistic bacterial infections has not been thoroughly investigated. This study aims to examine the effect of long-term PM exposure on *P. aeruginosa* infection in a murine model. **Objectives:** Determine whether long-term exposure to $PM_{2.5}$ enhances pulmonary infection with *Pseudomonas aeruginosa*, exacerbates lung tissue damage, and induces changes in specific immune response parameters within

the lung tissue of exposed mice compared to unexposed control animals. **Methods:** Eight weeks old BALB/c mice were grouped in: control, infection, PM, and PM + infection. Mice were exposed intratracheally to $PM_{2.5}$ (64 µg) for 28 days. After this time, mice were infected with *P. aeruginosa* (1.7 CFU/mouse). Subsequently, at day 3 and 7 post-infection, mice were euthanized; blood, lungs, and other organs were collected and stored until further analysis. **Results:** Long-term exposure to urban $PM_{2.5}$ resulted in significant weight loss in PM pre-exposed groups compared to controls. Additionally, we observed a higher bacterial load in the lungs of PM-exposed infected mice on days 3 and 7, compared to mice infected with *P. aeruginosa* alone. **Conclusion:** Long-term exposure to $PM_{2.5}$ of BALB/c mice favored *P. aeruginosa* infection, increasing the bacterial load in lungs of pre-exposed mice compared to unexposed controls. In addition, preliminary observations of the histological profile suggest an increase in inflammatory cell infiltration in the infection, PM and PM + infection groups on day 3.

252. 358. **CHEMOKINES CXCL9 AND CXCL10 PROMOTE B CELL DIFFERENTIATION INTO PLASMA CELLS AND IGG SECRETION IN COVID-19**

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Several studies have demonstrated an exacerbated humoral response in COVID-19 patients with acute respiratory distress syndrome (ARDS). These patients also developed a phenomenon known as “cytokine storm”, characterized by excessive pro-inflammatory cytokines and chemokines due to abnormal immune cell activation. While antibody production is crucial for viral clearance, its role in severe COVID-19 remains unclear. Although B cells require specific cytokines for activation and differentiation into antibody producing plasma-cells, the inflammatory factors driving the heightened humoral response in severe COVID-19 have yet to be identified. The aim of this study was to identify cytokine storm components positively correlated with the levels of anti-SARS-CoV-2 IgG in patients who had COVID-19, and then, evaluate the effect of these cytokines on plasma-cell differentiation and antibody secretion *in vitro*. Our results showed that at 4-months post-infection, IgG levels were significantly augmented in patients with ARDS. In addition, we found that chemokines CXCL9 and CXCL10 levels correlated significantly with IgG levels. Subsequently, a 3-phase protocol was used to differentiate human B cells in vitro in the presence or absence of CXCL9 and CXCL10. B cells activation was confirmed with CD86 and CD25 upregulation, whereas plasma-cell differentiation was confirmed with the presence of CD38hiCD27hi and CD138+ cells. The expression of the CXCL9/CXCL10 receptor (CXCR3) was also upregulated with the activation cocktail used in phase 1 and maintained during phase 2 and 3. We observed that while CXCL9 increased CD86 expression, the combined presence of CXCL9 and CXCL10 significantly increased the percentage of CD38hiCD27hi cells, CD138+ cells, and IgG secretion. Finally, we found that CXCL9 also enhanced CD40L expression on CD4+ T cells. Our findings reveal a critical role for CXCL9 and CXCL10 in plasma cell differentiation and IgG production. These chemokines appear to be key contributors to the exaggerated humoral response observed in severe COVID-19.

253. 361. **TISSUE-SPECIFIC ROLES OF REGULATORY T CELLS DURING CHRONIC EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION**

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We reported that after acute *Trypanosoma cruzi* (Tc) infection, Foxp3+ regulatory T cells (Tregs) frequency drops, facilitating the development of protective anti-parasite CD8+ T cell immunity. Additionally, we previously determined that during

chronic infection total and tissue repair (ST2+ KLRG1+) Tregs accumulate in skeletal muscle (SM), a Tc target tissue. Herein, we aimed at studying the role of Tregs in the chronic phase of Tc infection. For this, DEREG mice were infected with Tc and injected with diphtheria toxin (DT) to eliminate Tregs or PBS as control, either intraperitoneally (systemic depletion) or intramuscular (local depletion) at >100 days post-infection. Following systemic depletion, we found that DT treatment significantly increased the numbers of splenic Tconv cells and their activation/effector phenotype (CD44+, CD25+, CD107a+), while a marginal effect was detected on the splenic CD8+ T cell response. Interestingly, systemic depletion significantly reduced Tregs numbers in SM, coincident with a reduced activation status (CD44+, KLRG-1+) of CD8+ T cells in that tissue. To specifically evaluate the role of Tregs present in SM we performed local Treg depletion. DT injection significantly reduced both total and tissue repair Tregs numbers in SM. The major effect on effector immune populations was a tendency to decreased parasite-specific CD8+ T cells in mice that received DT, concomitantly with a significantly increase in SM parasite burden. Histological analysis revealed that perivascular leukocyte infiltration was more frequent in SM of DT- treated mice, where thrombosis and recanalization of vessels was detected in some cases. Altogether, our results suggest divergent roles of Tregs depending on their tissue of residence during chronic Tc infection. Tregs present in secondary lymphoid organs mainly suppress effector Tconv cells, while surprisingly Tregs present in SM may support parasite-specific CD8+ T immunity leading to an improved parasite control and milder tissue damage.

254. 363. **PATHOLOGICAL ROLE OF MO-MDSCS IN YERSINIA ENTEROCOLITICA- INDUCED REACTIVE ARTHRITIS**

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Background: monocytic myeloid-derived suppressor cells (Mo-MDSCs) are a population of immature myeloid cells with the ability to suppress T-cell responses. *Yersinia enterocolitica* (Ye) is a Gram-negative bacterium that causes reactive arthritis (ReA). The association between MDSCs and ReA has not been explored yet. **Objectives:** the purpose of this work was to investigate the Mo-MDSCs role in Ye-induced ReA. **Methods:** C57BL/6 mice were infected with Ye MCH-700 serotype O:3. The Mo-MDSCs were analyzed in regional lymphatic nodes to the joints (RLN) and in the spleen by flow cytometry. Furthermore, Mo-MDSC suppressor activity was evaluated in co-cultures with purified T cells. Nitric oxide (NO) and IL-17 levels were analyzed in culture supernatants. **Results:** we found that the percentage and number of Mo-MDSC increased in both RLN and spleen of infected mice that develop ReA, in contrast with uninfected mice ($p<0.001$, $p<0.01$ and $p<0.001$, $p<0.01$, respectively). It is noteworthy that the percentage of Mo-MDSC in RLN correlated with the arthritis score ($R2=0.9234$). Moreover, Mo-MDSC from the spleen of infected mice showed suppression activity on T cells. Also, NO production increased in the supernatants of Mo-MDSC/T-cell co-culture, but not in those of PMN-MDSC/T-cell ($p<0.01$), or MDSC depleted cells/T-cell ($p<0.05$). The analysis of Mo-MDSCs in RLN and spleen at different times post-infection (p.i.) demonstrated that these immune cells exhibited a marked increase on day 14 p.i ($p<0.001$ and $p<0.001$ respectively). Additionally, the supernatant of RLN cells obtained on day 21 p.i and stimulated with Ye antigens or concanavalin A (ConA) exhibited elevated levels of IL-17 in comparison to cells from control mice ($p<0.05$ and $p<0.0001$, respectively). Furthermore, *in vivo* MDSC depletion reduced the arthritis score. **Conclusion:** the presented evidence indicates that Mo-MDSCs may contribute to the pathogenesis of ReA.

255. 366. **ROLE OF PERTUSSIS TOXIN AND ADENYLATE CYCLASE TOXIN IN MODULATING MACROPHAGE PHENOTYPES DURING BORDETELLA PERTUSSIS INFECTION**

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During microbial infections, macrophages (MØ) can polarize into classically activated (M1) or alternatively activated (M2) cells. Previous studies demonstrated that M1 macrophages exhibit a stronger bactericidal response against *B. pertussis* compared to M2 macrophages. However,

although bacterial survival was higher in M2 macrophages, viable bacteria persisted in both phenotypes at later stages of infection. The present study investigates the roles of pertussis toxin (Ptx) and adenylate cyclase toxin (Cya) in modulating the M1 phenotype after infection. Flow cytometry analysis revealed two distinct macrophage populations 48 hours post-infection: one expressing high levels of M1 markers (CD80, CD40) and low levels of the M2 marker CD163, and another with the reverse profile. This result suggests that *B. pertussis* may drive a phenotypic shift from M1 to the more permissive M2 phenotype. Infections with Ptx- and Cya-deficient strains (Bp-ΔPtx, Bp-ΔCya, and the double mutant Bp-ΔPtxΔCya) confirmed that both toxins are critical in this modulation. Polymyxin B protection assays showed that the intracellular survival of Bp-ΔPtxΔCya was significantly lower than wild-type strain, indicating that Ptx and Cya enhance the survival of *B. pertussis* in M1 macrophages. Microscopic analysis showed that infection with the wild-type strain led to a reduction in M1-associated pseudopod elongation, a morphological change less pronounced in cells infected with the mutant strains. Furthermore, flow cytometry demonstrated a reduction in CD80 expression in wild-type-infected cells, a decrease not observed in cells infected with the mutant strains. Colocalization studies with the lysosomal marker Lysotracker revealed that bacteria surviving at 48 hours post-infection escape the lysosomal pathway. These results suggest that Ptx and Cya facilitate bacterial survival by shifting M1 macrophages towards an M2-like phenotype and enabling escape from the lysosomal pathway, highlighting the crucial role of both toxins in the modulation of macrophage phenotype during *B. pertussis* infection.

256. 371. **PLASMA LEVELS OF GALECTIN-1, 3, AND 9 AND ITS MRNA EXPRESSION ON PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH PULMONARY TUBERCULOSIS WITH OR WITHOUT TYPE 2 DIABETES MELLITUS AS AN ASSOCIATED COMORBIDITY**

Matilde Imhoff[1], Magdalena Diab[1], Ariana Díaz[1], Bettina Bongiovanni[1], Georgina Gallucci[1], Estefania Massa[1], Rocío del Valle Fernández[1], Marisa Derio[1], Susana Lioi[2], Diego Bértola[3], Natacha Maldonado[3], Natalia Garitta[3], Walter Gardeñez[3], Gabriel Rabinovich[4], María Luisa Bay[1], Oscar Bottasso[1], Luciano D'Attilio[1]

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Mycobacterium tuberculosis (Mtb) is the etiologic agent of tuberculosis, a disease that remains among the top causes of world death due to infectious agents. It is estimated that a quarter of the population is infected with *Mtb* and pulmonary form of tuberculosis (PTB) is the most frequent clinical manifestation of the disease. Type 2 Diabetes Mellitus (DM2) is a predominant factor in the host as it has shown it increases three times the risk of developing active PTB. In previous works, we have demonstrated that PTB patients showed an immune-endocrine- metabolic (IEM) imbalance: elevated plasma levels of pro and anti-inflammatory cytokines as well as cortisol, with diminished body mass index and dehydroepiandrosterone concentration. In these context, elevated circulating levels of galectins (Gal-1 and 3) were also found. These could be considered as another regulatory mechanism in immune response (IR) against Mtb. Regarding this, we aimed to study plasma levels of Gal-1, Gal-3 and Gal-9 as well as cytokines (IL-1β, IL-6, and IL-10). mRNA expression of Gal-1, Gal-3 and Gal-9 and some transcripts related to its mechanism of action (glycosyltransferases C2GnT1, GnT5, ST-6GAL1 and co-receptor TIM- 3), along with cytokines transcripts were also quantified in PBMCs from PTB and PTB+DM2 patients and their control groups (healthy controls-HCo, DM2). When measuring plasma levels of galectins all three showed elevated levels in PTB patients vs. HCo ($p<0.05$). In PTB+DM2 group differences were found also for Gal-3 vs. DM2 ($p<0.05$) and Gal-9 vs. PTB ($p<0.05$). Transcripts quantified for galectins, glycosyltransferases and cytokines were augmented in PTB patients vs. HCo ($p<0.005$, $p<0.05$, $p<0.05$, respectively). PTB+DM2 patients had augmented transcript expression of Gal-9, TIM-3 and IL-1β vs. HCo ($p<0.05$). Summarising, galectins could be another regulatory component along with IEM environment against Mtb in PTB and PTB+T2DM. Nevertheless, more studies are needed to elucidate this.

257. 372. **AEDES AEGYPTI SALIVARY PRO-**

TEINS AND THE MODULATION OF HOST INNATE IMMUNE CELLS IN CHIKUNGUNYA INFECTION

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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. Data were analyzed using a One-way ANOVA test and $p<0.05$ was considered statistically significant. 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. These findings suggest that AED7L2 and apyrase modulate the host immune response during CHIKV infection, with AED7L2 potentially reducing viral replication. Our preliminary results indicate that these salivary proteins could play a significant role in the pathogenesis of chikungunya virus infection.

258. 375. **TRYPANOSOMA CRUZI INFECTION IMPACTS GUT IMMUNE RESPONSE**

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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. Data were analyzed using a One-way ANOVA test and $p<0.05$ was considered statistically significant. 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. These findings suggest that AED7L2 and apyrase modulate the host immune response during CHIKV infection, with AED7L2 potentially reducing viral replication. Our preliminary results indicate that these salivary proteins could play a significant role in the pathogenesis

of chikungunya virus infection.

259. 393. **CLOSTRIDIOIDES DIFFICILE STAINING: A SIMPLE METHOD FOR FLUORESCENT DETECTION OF CLOSTRIDIOIDES DIFFICILE IN EX VIVO AND IN VITRO ASSAYS**

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Considered as an urgent global threat, *Clostridioides difficile* is a gram-positive, opportunistic potentially lethal pathogen that colonizes the large intestine. *C. difficile* has the ability to form resistant spores to survive but does not grow in the presence of oxygen. In fact, the use of fluorescent reporters and the design of genetic constructs is difficult due to their strictly anaerobic growth. Therefore, we aimed to perform a fluorescent staining for the vegetative and sporulated form of *C. difficile*, in order to use it in immunological functional assays.

Vegetative *C. difficile* was stained with fluorescein isothiocyanate (FITC) at 37 ºC for 1h; while for the sporulated form, malachite green (MG) dye was used at 70°C for 10 min. Staining efficiency and bacterial viability were evaluated by flow cytometry (FC), fluorescence microscopy (FM) and growth on BHI and CHROMagar™ *C. difficile* plates. FC showed 97% staining efficiency for FITC and more than 90% of viability for FITC-stained toxigenic and non-toxigenic strains. Staining *of C. difficile* spores with MG was analyzed by FC and confirmed by FM. For both stains, bacterial viability was validated by plate growth under anaerobic conditions. In addition, the interaction between stained bacteria and immune cells (peripheral blood mononuclear cells, monocytes, macrophages and/or platelets) was detected *in vitro*, as well as in a murine model of *ex vivo* cultured intestines. Furthermore, we observed stability of both stains in extended *in vitro* culture times (7 days) and after several thawing cycles. We have described a previously unreported, simple, fast and accessible method to obtain fluorescent *C. difficile*. Although further studies are required to explore their utility and potential, these stains can be applied in various assays to visualize *C. difficile* at the molecular and cellular level and better understand the host-pathogen interaction.

260. 395. **INCREASED EXPRESSION OF CHECKPOINTS IN NATURAL KILLER CELLS OF PATIENTS WITH ACTIVE LESIONS OF CUTANEOUS LEISHMANIASIS**

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Cutaneous Leishmaniasis (CL) is a public health problem, with many cases in Brazil. The disease is characterized by the appearance of skin lesions that can vary depending on the species of *Leishmania* as well as host factors. The immune response is directly linked to the course of the infection. Natural killer (NK) cells are known for killing infected cells without the need for prior sensitivity and for producing cytokines. However, there is still no consensus on their real role CLC. Therefore, we evaluate the percentage of NK and NKT cells in 11 patients with active CL lesions and 10 controls and investigated their expression of immune checkpoints. PMBC cells were isolated from their blood and labeled with antibodies anti: CD3, CD4, CD8, CD16, CD56, PD-1, TIGIT, CTLA-4 and

Granzyme b. Flow cytometer acquisition was performed (300,000 events/tube). To assess cytokine production, the cells were stimulated with total *Leishmania braziliensis* antigen. We observed a lower percentage of NK cells and double- negative (DN) NKT cells in patients with active lesions, while CD4+ NKT cells showed a higher percent-

age in this population. In addition, we observed greater expression of PD-1, Granzyme b, TIGIT and CLTA-4 in the NK cells. Significant production of IL-2, IL-4. IL-6, IL-10, IL-17, IFN-γ and TNF were also observed in patients with active lesions and after treatment, following stimulation with *L. braziliensis* antigen. Thus, we observed that patients with active lesions showed a decrease in the percentage of NK and NKT DN cells and an increase in the population of NKT CD4+ cells. In addition, we observed an increase in the expression of checkpoints, which may be jeopardizing the functionality of these cells, thus compromising the immune response in these patients.

261. 409. **LACK OF TLR9 EXACERBATES ALLERGIC-LIKE INFLAMMATORY RESPONSE IN THE LUNGS DURING EXPERIMENTAL INFECTION BY CRYPTOCOCCUS GATTII**

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Cryptococcosis is a potentially systemic mycosis responsible for approximately 625,000 deaths annually. *Cryptococcus neoformans* and *C. gattii* are the main etiological agents of cryptococcosis. Cryptococcal meningitis representing the most severe form of the disease. The literature indicates that an inflammatory response, characterized by eosinophilia, accumulation of Charcot-Leyden crystals, and elevated levels of IL-4 and IL-5, is observed in the lungs of mice inoculated with *C. neoformans*. Moreover, activation of TLR9, which recognizes CpG fractions of unmethylated ssDNA, reduced pulmonary eosinophilia and IL-5 production. However, the pulmonary inflammatory response during *C. gattii* infection remains poorly understood. Recently, our group demonstrated that TLR9 knockout (TLR9$^{-/-}$) mice are more susceptible to *C. gattii* infection. TLR9$^{-/-}$ mice have intense fibrosis, polymorphonuclear cell accumulation, and lower levels of IFN-γ and IL-17 in the lungs. We hypothesize that pulmonary *C. gattii* infection induces an allergic inflammatory response, which is exacerbated in the absence of TLR9. Hence, we intratracheally inoculated 10□ yeasts of *C. gattii* R265 strain in C57BL/6 WT and TLR9$^{-/-}$ mice. Our findings show that TLR9$^{-/-}$ mice have increased eosinophilia in the lungs three weeks after inoculation with *C. gattii*. Histopathological analysis revealed the accumulation of polymorphonuclear cells and Charcot-Leyden crystals in the lungs of TLR9$^{-/-}$ infected mice. Furthermore, the absence of TLR9 resulted in lower levels of IFN-γ, IL-6, and IL-17, and elevated levels of IL-4 and IL-5 in the lungs three weeks post-*C. gattii* infection. These results suggest that pulmonary *C. gattii* infection induces an allergic-like inflammation and that TLR9 is crucial for maintaining the Th1/Th17/Th2 balance. Our work contributes to the knowledge of the immunopathology of cryptococcosis. However, further studies are needed to elucidate the mechanisms involved in the immunopathogenesis of *C. gattii* infection.

262. 414. **ROLE OF PURINERGIC SIGNALING PATHWAY IN THE IMMUNE RESPONSE TO *TRYPANOSOMA CRUZI* INFECTION: EVIDENCE FROM TRANSCRIPTOMIC ANALYSIS OF HEART TISSUES FROM PATIENTS WITH CHAGAS CARDIOMYOPATHY**

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Background: The molecular mechanisms driving human chronic Chagas cardiomyopathy (CCC) remain elusive. Ischemic cells release ATP, triggering immune responses to inhibit infections, but it is quickly metabolized by CD73 and CD39 ectoenzymes into anti-inflammatory adenosine. Our research demonstrated that inhibiting CD73 during murine acute *T. cruzi* infection reduces

CCC progression and parasite load. Moreover, heightened CD73 expression in leukocytes from CCC patients' cardiac tissue correlates with myocarditis severity and parasite burden. **Objectives:** Our study aimed to explore the transcriptomic expression of purinergic pathway genes in left ventricular free wall heart tissue samples from CCC patients. Using the public RNA-seq database (GEO accession: GSE191081), we conducted a comprehensive analysis to investigate the potential mechanisms underlying CCC. **Methods:** Differentially expressed genes (DEGs) between CCC and control samples were identified using DESeq2, revealing disease-specific gene expression changes. We then performed Gene Ontology (GO) analysis with ClusterProfiler to categorize DEGs into relevant biological processes, molecular functions, and cellular components. To gain deeper insights, pathway enrichment analysis (PEA) with PathfindR highlighted key pathways involved in CCC pathogenesis. Finally, RNA-seq deconvolution using ADAPTS assessed differences in immune cell infiltration between CCC and controls. **Results:** CCC showed 2,544 DEGs (Padj<0.05, l2fc>|1|) compared to controls. GO-analysis identified purinergic receptor activity among the most enriched terms. PEA, using the KEGG database, placed the purinergic pathway in the top ten enriched pathways. NT5E was significantly upregulated in CCC. RNA-seq deconvolution revealed higher proportions of NK, CD8+, and CD4+ T-cells, and a lower proportion of M2 macrophages (P<0.01 for all comparisons) in CCC. **Conclusion:** The results show a significant differential expression of purinergic system components in CCC tissues that could
be related to the pathogenesis of the disease. The increased expression of the NT5E gene suggests a role for CD73 in the progression of CCC and parasite persistence in cardiac tissue.

263. 421. **A STEM-LIKE TCF1HI TISSUE-RESIDENT MEMORY CD8+ T CELL IN NON-LYMPHOID TISSUES**

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Background: T lymphocytes have the ability to form long-lived memory cells, giving rise to either circulating (T_{circ}) or tissue-resident (T_{RM}) populations. Circulating central memory (T_{CM}) cells retain stem-like potential for reexpansion and effector differentiation driven by the expression of "T Cell Factor 1" (TCF1). Conversely, T_{RM} cells are characterized as more terminally differentiated cells expressing the "Homolog of Blimp1 in T cells" (Hobit). Hobit has been suggested as a direct repressor of TCF1, whereby its expression in T_{RM} cells drives tissue-residency, effector differentiation and loss of stemness. Objectives: To investigate how expression of TCF1 and Hobit is regulated by antiviral CD8+ T cells establishing residency in different non-lymphoid tissues. Methods: Adoptive transfer of TCR-transgenic TCF1- and Hobit-reporter $CD8^+$ T cells into C57Bl/6 mice, followed by LCMV Armstrong infection and oral rechallenge with *Listeria monocytogenes* expressing LCMV gp33 (Lm-gp33). Statistics were calculated by performing unpaired t-test, One-way ANOVA test with Tukey's post-test or Two-way ANOVA test with Sidak's post-test. Results: We have identified a $TCF1^{hi}$ $Hobit^{hi}$ T_{RM} subset of CD8 T cells after acute viral infection that is distinct from established $Hobit^{hi}$ T_{RM} cells. $TCF-1^{hi}$ T_{RM} exhibit differential expression of integrins and cytotoxic molecules, and show a unique response-phenotype upon local reactivation in the gut following Lm-gp33 infection. We will discuss ongoing long-term depletion experiments of $TCF1^{hi}$ T_{RM} cells, testing their role in the replenishment of T_{RM} cells and their function in coordinating local recall responses. Conclusion: We hypothesize that the $TCF1^{hi}$ T_{RM} population could serve as a local reservoir of stem-like T_{RM} cells across tissues, being able to replenish and maintain the T_{RM} pool over time. Understanding the drivers of such heterogeneity highlights potential strategies to enhance long-lasting local immunity, such as mucosal vaccination and solid cancer T cell therapies.

264. 432. **EVALUATION OF THE CONTRIBUTION OF ECHINOCOCCUS GRANULOSUS LAMINATED LAYER COMPONENTS TO LOCAL IMMUNOSUPPRESSIVE EFFECTS**

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The larva of the cestode *Echinococcus granulosus* (hydatid) causes cystic echinococcosis, a chronic infection characterized by the parasite establishing strong immunoregulatory mechanisms. The hydatid is delimited by an acellular layer known as the laminated layer (LL), consisting of mucins and calcium deposits of *myo*-inositolhexakisphosphate (InsP6.Ca). The LL is the main structure exposed to host interaction. Moreover, during chronic infections, as the parasite grows, LL particles are shed from its outermost layers, potentially interacting with immune cells beyond the parasite surface. Thus, LL materials are good candidates to contribute to the immunoregulation mechanisms deployed by the parasite. Our group characterized the local immune environment established during the chronic phase of experimental echinococcosis in mice, identifying several indicators of an immunosuppressed milieu. To model chronic exposure to LL-derived materials, we repeatedly injected the peritoneal cavity of C57BL/6 mice with a preparation of LL particles (*wpLL*, whole particles of LL; 5 injections, twice a week). Twenty-four hours after the final injection, we observed local changes resembling those observed in chronic infection. These changes included the presence of M2-like macrophages (Relm-α+/Ym-1+/PD-L1+/PD-L2+), an expansion of regulatory (FoxP3+) and PD-1+ subpopulations of CD4+ T cells, and increased levels of IL-1Ra and TGF-β in the cavity fluid.
In this work, we aim to determine which of the LL components are contributing to the different effects in our experimental model. Then, we treated wpLL with EDTA, to chelate calcium and deploy the InsP6.Ca component (pLL) or with NaIO4 to oxidate and destroy mucin carbohydrate moieties (pLLIO4). Results indicate that none of the components are necessary to induce the local effects. We are now exploring whether protein moieties in the particles, such as host proteins adsorbed to the LL (mainly antibodies), or the non-glycosylated ends of the LL mucins, may play a role in the induction of wpLL effects.

265. 447. PERSISTENT IN VIVO EXPOSURE TO ECHINOCOCCUS GRANULOSUS LAMINATED LAYER: EVALUATION OF ITS IMMUNOSUPPRESSIVE IMPACT ON THE SYSTEMIC IMMUNE RESPONSE

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Introduction: *Echinococcus granulosus*, the causative agent of cystic echinococcosis, manipulates its host immune system in order to survive. During experimental infections (mouse peritoneal cavity), our group evidenced that the parasite larvae (hydatid) generates an immunosuppressed local environment (M2-like PD-L1/PD-L2+ macrophages, Treg cells expansion, TGF- beta and IL-1Ra production). The laminated layer (LL) is a massive structural component of the hydatid wall, exposed to interaction with the host immune system. Repeated injections of a LL preparation (wpLL, *from whole particles of LL*) in the peritoneal cavity mimicked the immunosuppressive environment observed during infections, suggesting the LL is mostly responsible for these effects. Objectives: To characterize the systemic response induced by repeated injections of wpLL and evaluate if wpLL injections can affect the immune response elicited against a second antigen (ovalbumin, OVA). Methods: Mice were injected 5 times with wpLL (or PBS), every 3 days, and then challenged with OVA administered together with alum (or alum alone as control), all intraperitoneally. Seven days after challenge, blood and spleens were collected and analyzed. Results and conclusions: Spleen T cell response was evaluated in terms of cytokine production after exvivo restimulation with wpLL, OVA or RPMI (control). Regarding T cell response to wpLL itself, a Th2 polarization was revealed (IL-5/IL-13/IL-10) when cells were kept in RPMI. This response was not altered on restimulation with wpLL, as if T cells were not able to respond to this stimulus. This suggests an hypo-responsive or even exhausted state of T cells. Regarding OVA, in our experimental conditions, wpLL injections did not affect T cell response to OVA restimulation; possibly the stimulus of alum is too strong, exceeding wpLL suppressive capacity. However, a slight trend towards a decreased production of OVA-specific IgG was observed in mice injected with wpLL. Currently, we are delving into the characterization of these observations.

266. 457. PLATELETS, ¿ACTIVE PLAYERS IN CLOSTRIDIOIDES DIFFICILE INFECTION?

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Clostridioides difficile (Cdiff) infection is the main cause of antibiotic-associated diarrhea. The need for new therapeutic strategies is imperative since *Cdiff* has been declared as an urgent threat among the antimicrobial's resistance threats. Therefore, treatments with focus on the host response could be promising. In this sense, platelets have sparked special interest due to their antimicrobial properties and their ability to modulate immunological functions. Here we evaluated the potential of platelets to recognize *Cdiff* and the ability to interact with immune cells. We obtained whole blood, platelet-rich plasma (PRP), washed platelets (WP) and monocyte-derived macrophages from healthy donors. The samples were stimulated with FITC- coupled live or dead vegetative *Cdiff* or the sporulated form stained with malachite green. Assays were performed using flow cytometry and fluorescence/confocal microscopy. In blood, vegetative *Cdiff* induces the formation of monocyte-platelet and granulocyte-platelet complexes, but not with lymphocytes. The spores also induce granulocyte-platelet complexes and interact with both monocytes and granulocytes. Platelets also form groups with macrophages and promote the internalization of vegetative *Cdiff*. Preliminary results showed that *Cdiff* spores can also be internalized. Platelets showed direct interaction with vegetative *Cdiff* both when WP and PRP were used in *in vitro* cultures, and this interaction increased at higher ratios of *Cdiff*. The spores- platelets interaction was MOI-dependent and was greater for PRP compared to WP, indicating that plasma components could modulate spore recognition. Moreover, a longer exposure time to PRP reduces the number of spores detected by flow cytometry, suggesting a microbicidal effect of platelets. Altogether, our results show that platelets can recognize *Cdiff* and interact with innate immune cells with the potential to kill this pathogen. These findings point out the importance of further exploring the use of PRP as a therapeutic agent for *Clostridioides difficile* infection.

267. 471. LAMINATED LAYER OF ECHINOCOCCUS GRANULOSUS: IN VIVO CHARACTERIZATION OF ITS EFFECTS ON THE LOCAL DENDRITIC CELL POPULATION

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Helminth parasites have co-evolved with their hosts' immune systems, developing diverse evasion mechanisms to ensure their survival. *Echinococcus granulosus*, a parasitic cestode, is the causative agent of cystic echinococcosis (hydatidosis). Its larval stage (hydatid) develops within the host's organ parenchyma shielded by a thick acellular layer called the laminated layer (LL). As the hydatid cyst grows, LL particles are released into the extracellular environment where they interact with the host's immune cells, suggesting a significant role of the LL in the parasite's immune evasion strategies. Previously, the investigation group developed a model of repeated injections of LL particles (wpLL), closely mimicking the immunosuppressive effects observed during experimental infection. Regarding local macrophage populations, this model showed that wpLL induced polarization into an M2-like phenotype with an immunosuppressive bias, evidenced by the expression of co-inhibitors PD-L1 and PD-L2. The objective of this study was to characterize the local dendritic cell (DC) populations within the wpLL model of repeated injections. Female C57BL/6 mice were injected intraperitoneally with wpLL or PBS (control) a total of five times over 2.5 weeks. One day after the last injection, peritoneal cells were recovered and analyzed. Statistical analysis was conducted using the Mann-Whitney test or Kruskal-Wallis test with Dunn's post-test. Our findings indicate that the different local DC subpopulations (conventional DCs or monocyte-derived DC) can internalize wpLL and become activated, increasing their expression of CD80 and CD86. This activation is skewed towards a phenotype with expression of CD206, Relm-α, and Ym1, and the immunosuppressive elements PD-L1 and PD-L2, similar to the one observed in macrophages. This suggests

that the DC could play an important role in the induction and/or maintenance of the immunosuppressed environment established by the parasite, highlighting the possible relevance of PD-L1 and PD-L2 expression in this phenomenon.

268. 473. **SEPSIS-INDUCED LIPID DROPLETS CONTRIBUTED TO LIVER DISFUNCTION AND TO RESISTENCE TO BACTERIAL INFECTION**

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Introduction: Sepsis is a life-threatening condition characterized by organ dysfunction due to a dysregulated host response to infection. Organ dysfunction is the most serious outcome of sepsis, directly linked to morbidity and mortality. Although sepsis alters lipid metabolism and increases lipid droplets (LDs), their role in tissue tolerance and organ dysfunction is not well understood. **Objective:** This study investigates the role of LDs in the pathophysiology of sepsis. **Methods:** Sepsis was induced in C57BL/6J mice via cecal ligation and puncture (CLP). We assessed hepatic steatosis, liver dysfunction, oxidative stress, inflammation, and bacterial load. To inhibit LD formation, mice were treated with A922500, a pharmacological inhibitor of DGAT1.
Results: We found that liver lipid droplet (LD) overload was significantly correlated with an increase in biomarkers of sepsis-induced liver dysfunction. Histological and lipidomic analyses revealed that between 24 and 48 hours, sepsis-induced hepatic steatosis progressed. Concurrently, we observed an increase in oxidative stress biomarkers, both in total liver tissue and within purified LDs. To determine whether this was directly related to steatosis, we inhibited LD formation by pharmacologically targeting the DGAT1 enzyme. This treatment prevented sepsis-induced LD accumulation, improved liver function, and reduced liver peroxidation and inflammation. However, DGAT1 inhibition did not lessen sepsis severity and, in fact, led to earlier sepsis-associated mortality. To further investigate this outcome, we examined the impact of DGAT1 inhibition on infection resistance mechanisms. DGAT1 inhibition increased bacterial load in the peritoneum and blood of septic mice at 6 and 24 hours post-sepsis. Additionally, the treatment inhibited LD accumulation in peritoneal cells and impaired the sepsis-induced production of nitric oxide (NO), PGE2, LTB4, CCL2, and IFN-β. **Conclusion:** Our findings indicate that while LDs play a role in controlling bacterial infections and modulating protective inflammation, they also contribute to liver dysfunction under severe inflammation and oxidative stress during sepsis.

269. 486. **HIGHER PREVALENCE OF SEVERE MYOCARDITIS IN MEN DOES NOT ACCOUNT FOR THE EXTENT OF FIBROSIS IN PATIENTS WITH CHRONIC CHAGAS CARDIOMYOPATHY**

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Background: Chagas disease, caused by *Trypanosoma cruzi*, constitutes a public health problem due to its high prevalence and the associated morbidity and mortality. Approximately 30% of infected individuals develop chronic myocarditis, which can lead to progressive heart failure and potentially require heart transplantation. Existing research suggests that most transplanted patients are males, though the reasons for this gender disparity remain unclear. **Objectives**: This study aimed to investigate whether there is a difference in myocarditis severity between men and women with chronic Chagas cardiomyopathy (CCC) and to compare the extent of fibrosis in cardiac tissue from patients with severe versus mild myocarditis accounting for sex differences. **Methods**: We examined 34 cardiac explants, which represented the total of CCC transplants performed at the Hospital Universitario Fundación Favaloro. Myocarditis severity was classified as severe, moderate, or mild based on qualitative criteria and confirmed by immunohistochemistry, considering the number of CD68+ plus CD3+ cells, as fol-

low: Severe≥median number; Moderate 25-50th percentile; or Mild≤25th percentile. Fibrosis was measured by Sirius Red staining, with images captured through a polarized light microscope for subsequent quantification. **Results**: Among 34 patients, 21 (61.8%) were male. Severe myocarditis was more prevalent in men (76.9%), while women had a higher rate of mild myocarditis (70%) (p = 0.039). Men had a significantly higher likelihood of severe myocarditis (odds ratio = 7.778, 95% CI = 1.11-38.62). However, there were no significant differences in fibrosis percentages between patients with severe (2.852 ± 1.609) and mild (2.574 ± 1.500) myocarditis, nor between men (3.246 ± 1.529) and women (2.287 ± 1.438). **Conclusion**: Our results support the association between male gender and increased severity of myocarditis in Chagas disease. However, the lack of significant differences in fibrosis suggests that the mechanisms driving myocardial damage in CCC may differ from those affecting fibrosis.

270. 491. **IMMUNOSENESCENCE PARAMETERS ANALYSIS IN PATIENTS WITH INFLAMMATORY CHRONIC DISEASES AS TUBERCULOSIS, AND ITS COMORBIDITY WHIT DIABETES MELLITUS TYPE 2**

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a leading infectious disease, particularly in low- and middle-income countries. Characterized by chronic respiratory symptoms, TB requires prolonged antibiotic treatment. Individuals with type 2 diabetes mellitus (DM2) are at increased risk of developing TB, as diabetes impairs immune function and exacerbates TB progression. Chronic inflammation, a key aspect of TB pathophysiology, may contribute to accelerated immunosenescence, a process involving adverse changes in leukocytes that impair their functional capacity, particularly during the development of adaptive immunity. To assess inflammation-associated aging, known as "inflammaging," we analyzed different contributing factors such as the senescence-associated secretory phenotype (SASP; pro- and anti- inflammatory cytokines IL-6, IFN-γ, IL-10), immune-endocrine imbalances (cortisol, DHEA-S), and senescence-related transcripts (HP-1, Sirt-3, ATM) in blood samples from patients with TB (n=45), TB-DM2 comorbidity (n=12), DM2 (n=12), and healthy controls (HCo, n=45). Consistent with previous results, TB and TB-DM2 patients exhibited higher plasma levels of pro-inflammatory cytokines compared to HCo ($p<0.01$). These patients also showed an immune-endocrine imbalance, characterized by elevated cortisol and decreased DHEA-S circulating levels. When analyzed by age groups (younger than 25 years, 25–50 years, and older than 50 years), as expected, DHEA-S levels decreased with age in HCo; however, this age-related decline was absent in TB patients, who presented low DHEA-S levels across all age groups ($p<0.05$). Additionally, senescence-related gene expression (HP-1, Sirt-3, ATM) was modified by age, with diminished expression in younger individuals. Particularly, TB patients showed decreased ATM mRNA levels in the middle age group (between 25 and 50 years; $p<0.05$) and increased HP-1 mRNA levels in those younger than 25 years ($p<0.01$). These findings suggest a differential behavior of these genes according to age. The high expression of HP-1, low expression of ATM, and low DHEA-S levels in younger TB patients may indicate an early senescence phenotype.

271. 496. **SINGLE-CELL RNA SEQUENCING AND MULTIPARAMETER FLOW CYTOMETRY DATA REVEAL HIGHLY CYTOTOXIC AND INFLAMMATORY PROFILE FROM GAMMA-DELTA T CELLS FROM CHAGAS DISEASE CARDIOMYOPATHY PATIENTS**

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Background: Chronic Chagas disease cardiomyopathy, the most severe consequence of *Trypanosoma cruzi* infection, is characterized by an intense mononuclear infiltrate that mediates damage in the heart tissue. By leveraging publicly available transcriptome data from heart tissue of patients with Chagas disease cardiomyopathy (CCC), we observed an upregulated γδ TCR gene expression, indicating that this cell subset might be important in the disease immunopathology. **Objective:** Therefore, we aimed to investigate the functional characteristics of γδ T- cells in CCC, focusing on the inflammatory and cytotoxic potential and *in vitro* responses, compared to chronic Chagas disease asymptomatic patients (IND) (CONEP2.809.859). **Methods and results:** Single-cell RNA sequencing analysis revealed a prominent cytotoxic and inflammatory potential in double-negative (DN) and $CD8^+$ γδ T cells from CCC patients, marked by high levels of message coding for cytotoxic molecules and NK-cell related receptors, whereas γδ T cells from IND were committed with general immune functions and apoptosis control. Using multiparameter flow cytometry, we demonstrate that in vitro stimulation of γδ T cells subsets from CCC with *T. cruzi* soluble antigen, promotes a pro-inflammatory profile characterized by elevated TNF-α and TNFR1 expression, as compared to IND. Notably, $CD8^+$ γδ T-cells from CCC express cytotoxic molecules that correlate with worse disease prognosis and display inflammatory cardiotropic chemotactic receptors, suggesting their potential recruitment to the heart. PCA and heatmap analysis using immune molecules expressed by γδ T- cells successfully segregated CCC and IND clinical forms. Myocardium gene expression analysis showed that γδ TCR gene in CCC positively correlates with inflammatory cytokines and CD8 gene expression, confirming their migratory and mainly inflammatory profile. **Conclusion:** This study suggests distinct activities of various subsets of γδ T cells in Chagas disease and their potential role in disease pathology, suggesting these cells as potential immunotherapy targets.

272. 498. EVALUATION OF DENGUE VIRUS REPLICATION IN PLATELET-MONOCYTE INTERACTION MODEL.

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Dengue virus (DENV) and host factors are responsible for disease pathogenesis, which vary from asymptomatic/mild infection to severe haemorrhagic fever. DENV can infect different cell types, such as monocytes and platelets. Monocytes can amplify DENV replication in antibody-enhanced infection. Platelets can replicate and translate DENV genome, however, viral replication is abortive. Platelets from dengue-infected patients are activated, apoptotic, secret pro- inflammatory mediators and express increased levels of P-selectin. These signals modulate platelet-monocyte interaction and induce inflammatory response in both cells. We aimed to evaluate if platelets can transfer newly synthesized viral RNA to monocytes during platelet- monocyte interaction and if it would trigger an inflammatory response in monocytes through dsRNA recognition by intracellular sensors. PBMCs and platelets were isolated from health donors (HD). Platelets were infected with DENV-2 and, after washing out the unbound viruses, cocultured with monocytes previously adhered in culture plates and treated or not with TLRs or RIG-I inhibitors. After 24, 48, 72h of interaction we quantified viral RNA and DENV replication in cell lysates and supernatants by qRT-PCR and plaque forming units (PFU) assay. Pro-inflammatory cytokines were measured by ELISA. Increased levels of DENV RNA were observed in infected- platelets lysates after 24 and 48h. Baseline levels of viral RNA were detected in monocytes cocultured with platelets. No infectious viral particles were detected in platelets or cocultures supernatants. The recognition of viral

RNA in monocytes cocultured with infected platelets led to increased secretion of CXCL8/IL-8 and CCL2/MCP-1, which were reduced when RIG-I downstream pathway was blocked. In conclusion, the transfer of DENV RNA from infected platelets to monocytes does not occur in a way that monocytes can replicate DENV genome and produce new virions. However, the transfer of viral RNA to monocytes contributes to the increased levels of inflammatory cytokines secretion through the activation of RIG-I/MDA-5 pathways.

273. 507. **INFLUENCE OF METABOLIC RHYTHMS ON TIME-DEPENDENT SEVERITY IN SEPTIC MICE**

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Sepsis, a leading cause of death from infection, is a syndrome caused by a dysregulated host response to pathogens. In murine models, sepsis mortality is strongly influenced by the circadian system: mice inoculated intraperitoneally with high doses of lipopolysaccharide (LPS) at the end of the day exhibit a higher mortality rate (~80%) than those inoculated at midnight (~30%). This is accompanied by greater inflammation and increased hypothermia. To investigate the mechanisms involved in this daily variation, we conducted proteomic analysis from serum samples taken 2 hours after LPS or vehicle administration at ZT11 (end of the day) and ZT19 (middle of the night) (ZT0: lights on; ZT12: lights off). This study revealed that proteins upregulated at ZT19 are associated with glucose metabolism, energy utilization, and lipid metabolism, whereas those differentially expressed at ZT11 are linked to inflammation, oxidative stress, cell communication, migration, and adhesion. To further study these differences we evaluated blood glucose levels after stimulation at both time points and the analysis showed that blood glucose levels significantly increased after LPS administration only at ZT11 but not at ZT19. To study how this glycemic response influences the severity in septic mice, we inhibited the hyperglycemic response administering metformin 30 minutes before LPS at ZT11, and we observed reduced severity levels in these animals. Conversely, administering exogenous glucose after LPS at ZT19, simulating the hyperglycemic response, also result in decreased severity. This result shows a different glucose metabolism at both time points in response to LPS, which generates early hyperglycemia associated with higher severity. In the resting phase, the metabolic state leads to an increase in blood glucose, contributing to a worse prognosis, while during the active phase, a more active glucose metabolism improves the prognosis.

274. 510. **MODERATE INFLAMMATORY PROFILE IN PATIENTS WITH CHIKUNGUNYA AND ZIKA VIRUS COINFECTION**

Lucas Sousa Magalhaes[1], Jaira Vanessa De Carvalho[2], Tatiana Rodrigues De Moura[2], Priscila Lima Santos[2], Regina Adalva De Lucena Couto Ocea[2], Angela Maria Da Silva[2], Camilla Natalia Oliveira Santos[2]

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The chikungunya (CHIK) and Zika (ZIK) viruses are mosquito-borne pathogens primarily transmitted by mosquitoes. The interplay between host immune response and viral activity is considered a crucial element in these differences among clinical manifestations. A major concern regarding the co-circulation of both viruses is the possibility of coinfection and its potential impact on patient health. This research is a cross-sectional observational study involving a case series of patients with concomitant infections of chikungunya and Zika viruses. Patients with acute arboviral disease, confirmed by RT-qPCR, were recruited and clinical information was obtained. A total of 45 immune mediators were quantified from sera using a multiplex assay grouped in: Th1/Th2 cytokines, inflammatory cytokines, chemokines, and growth factors. Regarding symptoms, coinfected patients showed no differences compared to those in the CHIK and ZIK groups. However, ZIK patients experienced a lower incidence of fever during the acute phase. Additionally, there were no differences in the presence of co-morbidities among the groups. None of the coinfected patients reported persistent symptoms after

the acute phase, like the mono-infected patients. The analysis revealed that 12 immunological mediators were differentially expressed in at least one patient group compared to healthy controls. Among these mediators, five showed elevated levels exclusively in CHIK patients compared to healthy controls (CCL2, CCL4, EGF, CXCL12, and IFN-α). Three mediators were highly expressed in both CHIK and co-infected patients (IL- 1RA, IL-8, and IFN-γ); three chemokines were elevated in all three groups (CCL5, CXCL1, and CXCL10), and one growth factor, SCF, showed elevated levels only in the ZIK group. The acute phase of coinfection with CHIK and ZIK led to a mild clinical symptoms like mono-infections, and present with an intermediate profile of circulating immune mediators between the two single infections groups.

275. 511. **ANALYSIS OF DIFFERENTIALLY EXPRESSED CHEMOKINES IN PATIENTS WITH VISCERAL LEISHMANIASIS BEFORE AND AFTER TREATMENT**

Lucas Magalhaes[1], Camilla Natalia Oliveira Santos[2], Maraiza Alves De Oliveira[2], Maria Clara Da Silva Cardoso[2], Sophia De Santana Ferreira Lemos[2], Maria Beatriz Monteiro Silva[2], Rogerio Silva Santos[2], Priscila Lima Santos[2], Roseane Nunes De Santana Campos[2], Tatiana Rodrigues De Moura[2], Angela Maria Da Silva[2]

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Visceral leishmaniasis (VL) is an infectious disease primarily affecting neglected populations and is caused by parasites from the genus Leishmania spp. The interaction between the vertebrate host's immune system and the parasites can lead to varying outcomes, ranging from asymptomatic contact to severe disease that may result in death. Chemokines are crucial components of the immune response and have been studied previously as factors that contribute to either parasite evasion or destruction. Consequently, this study aimed to evaluate the levels of chemokines in patients with visceral leishmaniasis. Patients with VL were recruited, and blood was collected at three time points: before treatment, and 30 and 180 days after the commencement of treatment. Individuals with a positive Montenegro skin reaction served as controls. Chemokines were measured using a multiplex kit according to the manufacturer's recommendations. Out of nine chemokines evaluated, seven were more highly expressed in patients before treatment and decreased over the course of treatment compared to controls: CCL2, CCL3, CCL4, CXCL1, CXCL8, CXCL10, and CXCL12. Similar levels of CCL5 and CCL11 were found in patients before treatment and in control donors, but levels of CCL5 were reduced at 180 days post-treatment compared to all other groups. Further analysis explored the chemokine levels from patients before treatment alongside hemogram data from a subgroup of patients. Correlation analysis showed that levels of CXCL1 are negatively correlated with the number of leukocytes, neutrophils, eosinophils, lymphocytes, and monocytes, while the number of circulating eosinophils was also negatively correlated with CXCL10, CCL2, CCL3, and CCL4. The sizes of the spleen and liver measured during VL diagnostics were not correlated with any of the chemokines evaluated in this study. These data reveal an intriguing relationship between CXCL1 and leukocyte recruitment, and the influence of chemokines on eosinophil levels in VL patients, meriting further investigation.

276. 512. **HOST RISK FACTORS AND IMMUNE RESPONSE: IMPACT ON DENGUE DISEASE PATHOGENESIS.**

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Background: Dengue continues being a significant cause of morbidity and mortality worldwide. Infection is produced by one of four viral serotypes and can be subclinical or cause a disease ranging from mild to a severe picture of shock and large hemorrhages with or without organ impairment. Uncontrolled immune response has been considered as a critical factor in the pathogenesis of Dengue severe disease, in which T memory cells and cytokines are pivotal. **Objectives:** To study the role of some host risk factors and the anti-viral T cells response on dengue disease pathogenesis are the aims of our study. **Methods:** Previous dengue immunity, genetic background and ethnicity are evaluated as risk factors for severity in dengue Cuban patients. Kinetic expression in PBMC of different genes (TNF-alpha, IFN-gamma, MIP1-alpha, TGF-beta, IL-10, FoxP3) was measured by RT-PCR in dengue patients with and without warning signs. Activation of T cell subsets was also studied by flow cytometry. PBMC from healthy dengue immune individ-

uals and controls were cultured with dengue virus simulating sequential infection, and was quantified the expression of cytokines, chemokines, transcription factors and cytotoxic markers genes by RT-PCR. **Results:** Higher TNF- alpha and IFN-gamma gene expression and higher counts of activated CD4+ and CD8+ T cells in the first four days after fever onset and higher expression of FoxP3 and IL-10 genes after the 4th day were associated to secondary dengue infection and warning signs. Heterologous sequential infection was associated with higher expression of pro- inflammatory cytokines, that was predominant in individuals with white skin color. **Conclusion:** Our results support the relationship between the cytokine levels and T cell activation with dengue severity. The memory T cell response lasts more than 20 years, which may lead to a long-term risk of serious disease. Ethnicity and genetic are demonstrated risk factors apparently linked to immune response.

277. 514. **ELEVATED MIF PLASMA LEVELS IN PEOPLE LIVING WITH HTLV-1: EXPLORING A PUTATIVE ROLE IN VIRAL PATHOGENESIS**

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3. (sin afiliación específica mencionada)

Background: Human T lymphotropic virus type-1 (HTLV-1) is a globally-spread retrovirus that, although mostly asymptomatic, may result in the development of adult T-cell leukemia/lymphoma (ATLL) or HTLV-1 associated myelopathy (HAM). MIF is a proinflammatory cytokine associated with Th17 cell imbalance in pathologic conditions. Here, we explore the role of MIF in the context of HTLV-1 infection. **Objectives:** To rule-out an association between MIF plasma levels, and phenotype and function of CD4+T- cell (CD4TC) subsets among people living with HTLV-1 (PLWHTLV). **Methods:** Blood samples were obtained from 12 healthy donors (HD), 10 asymptomatic, 7 ATLL and 9 HAM PLWHTLV. MIF, IL-8/10/17A and IFNγ in plasma were evaluated by ELISA. Expression of CD4, CD25, CCR4, IL17A, IFNγ, FoxP3 and CD74 (MIF receptor) was evaluated in lymphocytes by flow cytometry and FlowJo (10.10.0). Statistical analysis was performed using GraphPad Prism (8.4.3). **Results:** Plasma MIF was found to be significantly elevated ($p<0.0001$) in asymptomatics (median=85.8 ng/ml) and ATLL (median= 75.2 ng/ml), when compared to HD (median= 2.028 ng/ml) and HAM (median= 4.7 ng/ml). Percentages of IFNγ-expressing cells were significantly lower in bulk CD4+ ($p=0.0048$) and CD4+CCR4+ ($p=0.0468$) T-cell subsets in ATLL compared to asymptomatics. No significant differences were found in the percentages of IL17A-expressing cells among CD4TC subsets or study groups. Expression of IL-17A and CD74 were correlated within the CD4+CD25+CCR4+ subset in ATLL patients ($p=0.0155$, $r=0.9845$). No correlation was found between plasma MIF levels and CD4TC subsets. **Conclusion:** Our findings suggest that MIF may play a substantial role in the pathogenesis of HTLV-1, since it was elevated in PLWHTLV plasma. However, no association was found between MIF and CD4TC subsets in this exploratory analysis.

278. 515. **PERSISTENT CHRONIC CHIKUNGUNYA CHARACTERIZED BY RHEUMATOLOGICAL DISEASES IN PATIENTS IS ASSOCIATED WITH A ROBUST INFLAMMATORY PROFILE**

Camilla Natalia Santos[1], Jaira Vanessa de Carvalho[1], Tatiana Rodrigues de Moura[1], Priscila Lima Santos[1], Regina Adalva de Lucena Couto Ocea[1], Angela Maria da Silva[1], Lucas Sousa Magalhaes[2]

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The chikungunya (CHIK) virus is a re-emerging alphavirus that leads from mild limited clinical manifestations to persistent and debilitating chronic CHIK disease. Several studies suggest that the outcome of CHIKV infection is influenced by the host's immune response during viral infection. Thus, the current report aimed to evaluate immunological markers in patients with acute and chronic CHIK disease and to assess the clinical follow-up of patients with chronic CHIK disease. Participants were divided into two groups: the Acute-chikungunya group and the Chronic-chikungunya group. Levels of 45 cytokines, chemokines, and growth factors were assessed using a multiplex kit. Clinical and rheumatological evaluations were assessed during the study. The patients with Chronic disease were older than

those in the Acute group and reported more intense pain according to the visual analogue scale (VAS). Nine immune mediators were more highly expressed concomitantly in Acute and Chronic groups, CCL5, CXCL1, CXCL12, IL-1RA, IL-7, IL- 6, IL-8, Il-12p70, and IFN-γ. The Acute group exhibited elevated levels of CCL2, CXCL10, IFN-α, and IL-1RA. Conversely, nineteen mediators showed higher levels in the Chronic group, IL-4, IL-5, GM-CSF, CCL4, VEGF-D, EGF, FGF-2, PDGF-BB, IL-1α, Il-15, IL-31, LT-α, IL-10, IL-27, IL-9, IL-21, IL-22, IL-23, and IL-17A. In the cohort analysis, all patients in the Acute group were discharged after the first medical evaluation without signs of chronic progression. The chronic patients displayed symptoms such as general arthralgias, tendinitis, spinal disorders, fibromyalgia, and inflammatory arthritis. Two regression survival analyses were performed, and the results indicate that elevated levels of IL-23, the presence of inflammatory arthritis, and higher VAS scores are associated with an increased risk of prolonged and persistent chronic chikungunya disease. Conversely, the presence of FGF-2 and IL-4, along with a diagnosis of arthrosis and positive IgG anti-chikungunya, are associated with a protective effect.

279. 519. DIFFERENTIAL EXPRESSION OF GROWTH FACTORS IN PATIENTS WITH VISCERAL LEISHMANIASIS

Camilla Natalia Santos[1], Lucas Sousa Magalhaes[2], Maraiza Alves de Oliveira[1], Maria Clara da Silva Cardoso[1], Sophia de Santana Ferreira Lemos[1], Maria Beatriz Monteiro Silva[1], Rogerio Silva Santos[1], Priscila Lima Santos[1], Roseane Nunes de Santana Campos[1], Tatiana Rodrigues de Moura[1], Angela Maria de Oliveira[1]

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Visceral leishmaniasis (VL) is a serious disease caused by Leishmania spp. parasites that can be fatal if untreated. The immune response plays a crucial role in controlling the parasite, although the parasite can deploy various mechanisms to evade this response. Growth factors are vital components of the immune system, and some have been previously associated with VL, although the information available is limited. This research aimed to evaluate the levels of growth factors in patients with VL. Patients were recruited during VL diagnostics, and samples were obtained at three points: before treatment (D0), 30 days after treatment, and 180 days after treatment. Patients with a positive reaction in the Montenegro skin test served as controls. A multiplex assay was performed to measure growth factors in the patients' sera. Out of ten growth factors measured, five exhibited differential expression in VL patients. Higher levels of stem cell factor (SCF) were observed in patients both before and immediately after treatment. Placenta growth factor-1 (PlGF-1) and nerve growth factor-beta (NGF-beta) showed elevated levels in VL patients both before and after treatment compared to the control group, while levels of hepatocyte growth factor (HGF) decreased by the end of treatment compared to levels measured before and during treatment. Conversely, fibroblast growth factor- 2 (FGF-2) showed increased levels only 180 days after treatment. The immune mediator's vascular endothelial growth factor A, epidermal growth factor, and platelet-derived growth factor BB maintained similar levels across all comparisons. The analysis was unable to detect levels of brain-derived neurotrophic factor and vascular endothelial growth factor D in most patients. The results of this research demonstrate that growth factors are differentially regulated during VL, suggesting that further studies are necessary to understand the underlying mechanisms more comprehensively.

280. 530. STUDY OF THE ANTIMICROBIAL AND INMUNOMODULATORY CAPACITY OF CELL WALL AND PEPTIDOGLYCAN OF LACTIPLANTIBACILLUS PLANTARUM ON STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM DIABETIC FOOT ULCERS

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The antimicrobial properties of the cell wall (Pc) and peptidoglycan (Pg) of *Lactiplantibacillus plantarum* (Lp) were studied on the virulence factors of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from diabetic foot ulcers and the microbicity activity of polymorphonuclear (PMNs) infected with MRSA. Diabetic patients

with ulcers were treated with a topical application of Lp. Bacteriology techniques were applied for the isolation and identification of MRSA strains. PCR was utilized to determine fnbA,sdrC, eap, emp, cna, clfA genes expression .The Pc and Pg capacity on the inhibition of the MRS biofilm was studied. PMNs isolated from patients were stimulated with Pc and Pg and microbicidal activity, phagocytosis and NETosis were measured. An increse in the expression of the eap, fnbA and clfA genes was observe in the MRS strains obtained from the biopsies. All MRS strains isolated formed biofilm. Pc and Pg inhibited the MRSA biofilm after 18 h of incubation. Significant differences were observed between the MRSA biofilm without treatment and the biofilm treated with Pg (100% vs 35%<0.005) and Pc (100% vs 40% <0.005). Increased phagocytic activity of PMNs was observed in healthy patients only stimulated with Pg (230 ifm vs 402 ifmp <0.001). The same was observed for diabetics patients PMNs (210 ifm vs 460 p <0.001). The PMNs of healthy patients showed greater induction of NETosis (4000 RFU) vs PMNs of diabetics patients (2000 RFU). A significant increase in NETosis of PMNs of diabetics patients stimulated with Pg was observed (6000 RFU). A decrease in UFC/ml was observed in the PMNs of diabetic patients stimulated with Pg compared with those no stimulated ($2x10^6$ vs $6x10^6$ p <0.01). Pg inhibit the biofilm of MRS strains and stimulates the process of phagocytosis, netosis and microbicial activity of PMNs from diabetic patients and favors the control of MRS infection. Diabetic foot infections and delayed wound healing are suggested as the major therapeutic difficulties associated with diabetic foot ulcer, due to the increasing global antimicrobial grugs resistance issues. In previous studies we showed that topical application of *Lactobacillus plantarum* (Lp) culture favored the healing process. We investigated the effect of Lp and culture supernatant on the microbicidal activity of polymorphonuclear cells (PMNs) infected with *Staphylococcus aureus* (*S. aureus*). **Material and methods:** Diabetic patients with ulcers were treated with a topical application of Lp. The study was approved by the Hospital Ethics Committee. Biopsies were taken at day 0, 10 and 30 post-treatment and histopathological and bacteriological studies were performed. At the same time, polymorphonuclear cells (PMNs) were isolated from the circulating blood of the patients. PMNs were stimulated with Lp and culture supernatant to perform the following tests: phagocytosis by flow cytometry, NETosis by fluorometry and microbicidal activity. Methicillin-resistant *Staphylococcus aureus* (*S. aureus*) isolated from ulcers was used as infectious strain in all tests. **Results:** PMNs from healthy patients was used as control group. Increased phagocytic activity of PMNs was observed in healthy patients only stimulated with Lp (230 ifm vs 402 ifm p <0.001). The same was observed for diabetics patients PMNs (210 ifm vs 460 p <0.001). The PMNs of healthy patients showed greater induction of NETosis (4000 RFU) vs PMNs of diabetics patients (2000 RFU). A significant increase in NETosis of PMNs of diabetics patients stimulated with Lp was observed (6000 RFU). A decrease in UFC/ml was observed in the PMNs of diabetic patients stimulated with Lp compared with those no stimulated ($2x10^6$ vs $6x10^6$ p <0.01).
Conclusions: Lp stimulates the process of phagocytosis, netosis and microbicial activity of PMNs from diabetic patients and favors the control of *S. aureus* infection.

281. 533. ANALYSIS OF PLATELET ACTIVATION AND INTERACTION WITH EPITHELIAL CELLS DURING SARS-COV-2 INFECTION

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Background: Platelets are essential for hemostasis and thrombus architecture. In addition, they can recognize viral pathogens and mediate the inflammatory response. During SARS- CoV-2 infection, platelets are activated, contributing to hypercoagulability and inflammatory amplification, which can exacerbate pulmonary vasculature injury and induce immunothrombosis. Therefore, it is crucial to understand the role of platelets in SARS-CoV-2 infection and their interaction with lung epithelial cells. **Objectives**: To characterize platelet activation and interaction with pulmonary epithelial cells during SARS-CoV-2 infection, and to elucidate the mechanisms and immunometabolic changes resulting from this interaction. **Methods**: Epithelial lung adenocarcinoma cells (ATCC/HTB-55, Calu-3) were infected with SARS-CoV-2 in the presence or absence of platelets from healthy donors. The interaction be-

tween these cells was evaluated 24 and 48 hours post-infection. **Results**: Platelets are activated by SARS-CoV-2 infection but do not generate infectious viral particles. However, platelets induce increased viral replication in infected Calu-3 cells and increase the production of thromboxane A2 and tissue factor. Inhibition of platelet activation pathways and thromboxane synthesis led to reduced viral replication. Amplification of immunometabolic changes and non-inflammatory cell death was observed during the interaction between platelets and infected Calu-3 cells. **Conclusion**: Platelets modulate the response of infected Calu-3 cells, leading to increased viral replication without progressing to apoptotic cell death. The data suggest that viral replication is enhanced in the presence of platelets, driven by increased release of thromboxane and tissue factor from platelets, which contribute to platelet activation and aggregation, thereby collaborating with thromboinflammation.

282. 543. **CAPTURE OF CIRCULATING PARASITE MUCINS BY KUPFFER CELLS IN CYSTIC ECHINOCOCCOSIS: COMPARISON BETWEEN THE MUCIN INJECTION AND EXPERIMENTAL INFECTION MODELS.**

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Cystic echinococcosis is caused by the larval stages (hydatids) of cestode parasites belonging to the species cluster *Echinococcus granulosus* sensu lato. Hydatids are bladder-like structures that attain large sizes within internal organs of livestock ungulates and humans. Hydatids are protected by the massive acellular laminated layer (LL), composed mainly by mucins. Parasite growth requires shedding of LL materials. We previously published that in experimental (intraperitoneal) mouse infections, LL materials circulate and are selectively captured by the liver Kupffer cells (KC), in a manner mostly dependent on the C-type lectin Clec4F. We also reported similar observations after intraperitoneal injection of a particulate LL mucin preparation (pLL), and analysis 24 h later. Now we have compared these two models in terms of (i) compensatory uptake of LL materials by non-liver organs in Clec4F gene-deficient (KO) mice, (ii) KC responses in terms of the checkpoint molecule PD-L1, proposed to be involved in KC-dependent liver tolerance. In both models, LL materials were detected in the spleen (white and red pulp) and mesenteric lymph nodes, but only after pLL injection was stronger uptake detected in Clec4F KO than in WT mice, perhaps reflecting the different kinetics of the models. In infected mice but not after pLL injection, we detected PD-L1 upregulation in KC, and this correlated with LL material uptake across the two mouse genotypes studied. KC are known to up-regulate PD-L1 when they present antigen to specific $CD4^+$ T cells. Our observations thus probably reflect that in the (chronic) infection model, KC present antigen to frequent, previously primed $CD4^+$ T cells whereas in the pLL injection model $CD4^+$ cells capable of interacting specifically with KC are few. This suggests that Clec4F-dependent LL material uptake by KC leads to antigen presentation, which we reason may be linked to the generation of tolerance towards parasite antigens.

283. 550. **ROLE OF GLUCOCORTICOID RECEPTOR EXPRESSION IN CHRONIC CHAGAS CARDIOMYOPATHY: IMPLICATIONS FOR SYSTEMIC INFLAMMATION AND CARDIAC HYPERTROPHY**

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Chronic Chagas cardiomyopathy (CCC) is a disease caused by the *T. cruzi* parasite. In previous studies, we demonstrated that a systemic inflammatory state was evident in these patients, as indicated by increased levels of IL-6 and TNF-α in circulation, along with hormonal abnormalities characterized by decreased DHEA-S and cortisol levels, and an imbalanced cortisol/DHEA-S ratio. In this context, we propose that altered regulation of glucocorticoid (GC) and GC-receptor (GR)-mediated circuits may be failing to control

systemic inflammation. Additionally, the involvement of GR and its isoforms in these circuits has been unexplored. Therefore, we investigated the potential influence of GC response regulation in Chagas patients and its possible implication in CCC pathogenesis, particularly in cardiac tissue. CCC patients, ischemic cardiomyopathy patients (ICM), and *T. cruzi*-seronegative individuals (Co) were recruited at Centenario Hospital, Rosario, Argentina. The expression of GR-α/β isoforms, 11β-hydroxysteroid dehydrogenase type-1 (11b-HSD1), cytokines, and tristetraprolin (TTP) were analyzed in PBMCs using RT-qPCR. Additionally, GR immunoreactivity in explanted chagasic hearts was evaluated. GR-α was similarly expressed in PBMCs from Co and CCC individuals, but 11β-HSD1 expression was increased only in CCC (CCC vs. Co, CCC vs. IMC; $p<0.05$), accompanied by elevated levels of IL-6 and IFN-γ, and higher IL-6/TTP and IFN-γ/TTP ratios (CCC vs. Co, CCC vs. IMC; $p<0.05$). Inflamed myocardium from CCC patients showed higher GR expression than other groups (CCC vs. Co, CCC vs. IMC; $p<0.05$). Furthermore, cardiomyocyte diameter and nuclear area were significantly increased, particularly in CCC hearts (CCC vs. Co, CCC vs. IMC; $p<0.05$), with most hypertrophied cardiomyocytes exhibiting greater immunoreactivity for GR (GR+ diameter/area vs. GR- diameter/area; $p<0.05$). In conclusion, CCC appears to be associated with increased GR expression in cardiac tissue, providing a basis for further studies aimed at elucidating the influence of GR expression and function on CCC pathophysiology and cardiomyocyte hypertrophy.

284. 561. **NEUTROPHIL PROFILES OF SEVERE COVID-19 PATIENTS POST-VACCINATION**

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Background: A dysregulation in the innate immune response against SARS-CoV-2 may contribute to a worse prognosis in COVID-19. Different functional profiles of neutrophils have been described, playing a crucial role in antiviral defense and in the inflammatory process triggered by the virus. With the advancement of vaccination, understanding how cells and immune mediators contribute to the pathogenesis or protection of COVID-19 is essential. **Objectives**: Thus, it was aims to evaluate the role of neutrophils and their mediators in severe COVID-19 post-vaccination. **Methods:** An observational case-control study was conducted using data, sera, and circulating neutrophils from patients with severe COVID-19. sTREM-1 and IL-6 serum levels were quantified by ELISA. Neutrophils were extracted from peripheral blood samples and subjected to flow cytometry experiments to evaluate the frequency and expression of surface receptors related to suppressive, antigen presentation, cytotoxic, phagocytosis and inflammatory activities. Comparisons between groups and correlations among immune and clinical variables were performed. **Results**: Serum levels of IL-6 were significantly higher in severe COVID-19 unvaccinated patients. In severe COVID-19 vaccinated patients, neutrophils expressed more TREM-1, CD182, HLA-DR, PD-L1, and HLA-DR MFI surface markers compared to severe COVID-19 unvaccinated patients. Additionally, neutrophils from severe COVID-19 vaccinated patients showed an increased percentage in the CD16+CD182+-TREM-1+ and HLA-DR+PD-L1+ populations. Correspondingly, severe COVID-19 vaccinated patients demonstrated significant correlations primarily related to the number of PD-L1+ neutrophils and inflammatory cytokines. **Conclusion** Together, the data presented here suggest that vaccination may be associated with a more robust and regulated neutrophil response during SARS-CoV-2 infection.

285. 564. **STUDY OF THE IMPACT OF LATENT HERPES SIMPLEX VIRUS TYPE 1 INFECTION ON SEPSIS SEVERITY**

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Sepsis is defined as a life-threatening organ dysfunction disease caused by a dysregulated host response to an infection. This pathology is considered an important global health problem with high morbidity and mortality rates, despite extensively studied. The septic syndrome is mainly caused by bacterial infection and its products, as endotoxins, producing endotoxemia which enhances production and release of proinflammatory cytokines increasing tissue and organ damage. However, the severity of sepsis syndrome also depends on other host factors, such as genetics, age and comorbidities. Recent studies suggest that concomitant latent virus infections may contribute to disease severity. Viral loads from herpesviruses in the blood, such as herpes simplex virus type 1 (HSV-1) is associated with immunosuppression and more days in intensive unit care during prolonged sepsis. However, whether previous HSV-1 infection relates to increases sepsis severity its unknown. We evaluated the effect of endotoxemia by LPS injection after asymptomatic HSV-1 brain infection on the onset and severity of sepsis in C57BL/6 mice. Infected and uninfected mice were subjected to endotoxemia by LPS intraperitoneal injection. Importantly, we observed more severe sepsis and increased mortality in mice previously infected with HSV-1. Biochemical parameters in blood and cytokine RNA levels in tissue were also measured. Our results support the notion that previous infection with HSV-1 enhances sepsis severity, which could be an important factor in morbidity and mortality in sepsis.

286. 566. ASSOCIATION OF ALLELIC AND GENOTYPIC FREQUENCIES OF SNP RS2234246 IN THE TREM-1 GENE IN VISCERAL LEISHMAN-IASIS INFECTION

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Introduction: Visceral leishmaniasis (VL) is a parasitic, systemic, and potentially fatal disease for humans. The activation of phagocytes is crucial in the host response against the parasite, and their functions are modulated by the expression of cell surface receptors. TREM-1 is an important cell surface receptor in the amplification and regulation of the inflammatory response. **Objective:** To evaluate the single nucleotide polymorphism rs2234246 in the TREM-1 gene in patients with visceral leishmaniasis. **Methodology:** This case-control study is being conducted at the Laboratory of Immunology and Molecular Biology of the Federal University of Sergipe. The case group consists of patients clinically and laboratory-diagnosed with VL, and the control group includes healthy contacts residing in endemic areas for VL. All donors were from the state of Sergipe and were included in this study from 2011 to 2019. The TREM-1 SNP rs2234246 (C>T) was genotyped using a TaqMan® probe by qPCR. Subsequently, the dosage of soluble TREM-1 will be quantified in serum through Enzyme-Linked Immunosorbent Assay (ELISA). **Results:** Ninety-four patients were included in the case group, and 127 in the control group. These were subdivided after the results of the delayed-type hypersensitivity skin test (DTH) into DTH+ (n=28) and endemic control (EC; n=99). Initial analysis showed that genotypes with at least one C allele (CC+CT) were more frequent in the DTH+ group when compared to the case group, in which the TT genotype was more frequent (OR=3.887, 95% CI = 0.98 – 17.59, p=0.09). **Conclusion:** Our preliminary results suggest that the TT genotype, already associated with higher levels of TREM-1 gene expression and soluble TREM-1, may be involved in the pathogenesis of VL. The more severe cases of VL are clinically related to an exacerbated inflammatory response, which is consistent with our results.

287. 567. DEVELOPMENT OF A NUCLEOPROTEIN-SPECIFIC HUMANIZED MONOCLONAL ANTIBODY THAT PROTECTS AGAINST RESPIRATORY SYNCYTIAL VIRUS INFECTION

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Background: The human Respiratory Syncytial Virus (hRSV) is a pathogen of global concern,

causing seasonal epidemics associated with significant morbidity and mortality, particularly in preterm infants and older adults. All licensed monoclonal antibodies (mAbs) developed against hRSV are based on targeting the hRSV fusion protein (F-hRSV). The most used mAb, Palivizumab, is a high-cost drug with moderate effectiveness. Meanwhile, the recently approved anti-F-hRSV mAb, Nirsevimab, has modifications in its Fc (fragment, crystallizable) region, leading to a significantly increased half-life. In our laboratory, we have identified that the hRSV nucleoprotein (N-hRSV) is expressed on the surface of hRSV-infected cells therefore, it could be considered an interesting target against infection by this virus. **Objectives:** This work seeks to develop humanized anti-N-hRSV mAbs and evaluate their efficacy in protecting against hRSV infection. **Methods and Results**: We developed four clones of humanized anti-N-hRSV mAb (P1-04H, P1-05D, P2-01A, and P2-01D), which were then manufactured under GLP conditions. These four clones of humanized anti-N- hRSV mAb were purified and showed high affinity for N-hRSV, as determined by SDS-PAGE and surface plasmon resonance, respectively. Moreover, using BALB/c mice as an animal model for infection, we observed that treatment with humanized anti-N-hRSV mAbs significantly reduced the pathology associated with hRSV infection, as evidenced by reduced viral loads in the lung and decreased neutrophil infiltration in the bronchoalveolar lavage (BAL). Furthermore, *in vitro* analyses showed that the four clones of humanized anti- N-hRSV mAb displayed antibody-dependent cell cytotoxicity (ADCC) and complement- dependent cytotoxicity (CDC) activity. Additionally, the pharmacokinetic analyses in BALB/c mice immunized with the four clones of the humanized anti-N-hRSV mAbs showed that the four clones exhibited differential pharmacological profiles, wherein P2-01A and P2-01D clones were detectable in the blood after 30 days post-immunization. **Conclusion:** All four clones of humanized N-hRSV-specific mAbs displayed promising preclinical profiles by protecting against hRSV infection.

288. 574. **PURINERGIC SIGNALING IN *CRYPTOCOCCUS NEOFORMANS* INFECTION: MOLECULAR AND PHARMACOLOGICAL STUDIES.**

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Cryptococcosis is a systemic fungal disease caused by *Cryptococcus neoformans*. The infection initially occurs in the lungs and can spread to other organs. Currently, there is no efficient treatment for late-diagnosed cases, necessitating new therapeutic targets. Purinergic signaling is a pathway activated by extracellular nucleotides and nucleosides, which are relevant in initiating and maintaining inflammatory responses against pathogens. However, the role of purinergic signaling in cryptococcosis is unclear. Thus, this work explores the possible implications of purinergic signaling in *C. neoformans* infection. For this sutudy, female Balb/c mice (6-8 weeks old) were divided into two groups: Sham and infected (H99). Each group was further subdivided into two subgroups: treated with PBS (SHAM and H99) and Brilliant Blue G (BBG) (SHAM-BBG and H99-BBG), a P2X7 receptor antagonist. On day 0, H99 groups were intratracheally instilled with $1x10^5$ *C. neoformans var. grubii H99*. On days -1, 2, and 5, BBG (50 mg/kg) was injected intraperitoneally. On day 7, mice underwent lung mechanics analysis, followed by euthanasia for lung and brain collection for molecular evaluation. Compared to Sham, the H99 group showed increased lung elastance, pulmonary resistance, and mRNA levels of purinergic receptors P2X7, P2Y2, and P2Y12 in the lung. Additionally, increased mRNA levels of pro- inflammatory cytokines (TNF-alpha, IL-1beta, IL-6, and IFN-gamma) were observed in the lung. BBG treatment reduced mRNA levels of P2X7 and pro- inflammatory cytokines (TNF-alpha, IL-1beta, IL-6, and IFN-gamma) in the lungs of infected animals. Conversely, BBG treatment increased mRNA levels of pro- inflammatory cytokines in the cortex (IL-1beta) and hippocampus (TNF-alpha and IFN-gamma) and reduced the survival time of infected animals. These results suggest P2X7 inhibition reduces lung inflammation but facilitates *C. neoformans* spread to the brain. Ongoing experiments will clarify this hypothesis and the role of purinergic signaling in *C. neoformans* infection.

289. 575. **CHARACTERIZATION OF THE SPECIFIC IMMUNE RESPONSE TO *T. CRUZI* IN PREGNANT WOMEN**

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Vertical transmission of Trypanosoma cruzi can occur during pregnancy of an infected person, either in the acute or chronic stage. It is an epidemiologically important route, especially in non-endemic areas, and cannot be prevented during pregnancy since trypanocidal treatment cannot be administered during this stage. Although the mechanisms involved are unknown, it has been established that it is a complex phenomenon involving the maternal-fetal-placental unit, the immune response of the pregnant woman, the fetus, and also the characteristics of the infecting parasite. In the present work, our objective was to characterize the CD4+ T response specific to T. cruzi during pregnancy. For this purpose, peripheral blood mononuclear cells were isolated from chronically-infected and from uninfected pregnant women. As an antigen to stimulate CD4+ T cells, we used lysates of parasites from vertical transmission isolated by blood culture in our laboratory. After in vitro stimulation, the production of cytokines IFN-gamma, IL-2, TNF-a, IL-17 and IL-10 were detected by intracellular staining by flow cytometry. We found that CD4+ T lymphocytes from infected-pregnant women respond differently to the lysates derived from T. cruzi. This allows us to infer that there are antigenic differences in the infecting parasites that would induce a maternal immune response with different degrees of protection for vertical transmission. In addition, we observed high levels of IL-2, TNF and IL-10 production specific for T. cruzi in two uninfected pregnant women from an endemic area, which shows that they were likely to have been previously exposed to T. cruzi and still maintain memory T cells. These results contribute to an area not yet explored to provide knowledge about the immunological profile of chronically infected women in relation to pregnancy and to vertical transmission of the parasite.

290. 584. **ROLE OF CYCLOOXYGENASES 1 AND 2 IN HERPES SIMPLEX TYPE 1-INFECTED DENDRITIC CELLS**

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Herpes simplex virus type 1 (HSV-1) is a prevalent microorganism that produces lifelong infections in humans by establishing latency in sensory and autonomic neurons. HSV-1 also infects dendritic cells (DCs), which are professional antigen-presenting cells that initiate and regulate antiviral immune responses. Importantly, HSV-1 negatively modulates DC function and ultimately kills these cells. Cyclooxygenases (COXs) are host enzymes that metabolize arachidonic acid into prostaglandin G2 (PGG2), which is subsequently converted into prostaglandin H2 (PGH2). PGH2 acts as a precursor of PGE2, PGD2, and PGI2 synthesis, as well as thromboxane (TXA2), which are involved in inflammatory and non-inflammatory processes. Previous reports indicate that COX-2 products can suppress the functions of DCs. Here, we explored the role of COXs in the modulation of DC function after infection with HSV-1. We found that HSV-1 infection significantly modulates the expression of COX-2 in infected DCs, as determined by RT-qPCR and Western Blot, and that the pharmacological inhibition of COX-2 recovers the viability of DCs infected with this virus, modulates their cytokine profile and maturation, altogether promoting T cell activation. Interestingly, the pharmacological inhibition of COX-2 did not impact the yield of HSV-1 virions from DCs. These results indicate that HSV-1 induces COX-2 expression in DCs, which relates to the death of these cells. Authors are supported by the Millennium Institute on Immunology and Immunotherapy #ICN2021_045.

291. 599. **CORRELATION OF CARBOXYPEPTIDASE A3 MAST CELLS AND LUNG FIBROSIS FOLLOWING SARS-COV-2 INFECTION**

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Background: COVID-19, caused by SARS-CoV-2, induces an uncontrolled immune response that leads to excessive inflammation, damaging lung parenchyma. Mast cells (MC) are well-known for their involvement in inflammatory conditions. Previous research has linked MC carboxypeptidase A3 (CPA3) to severe COVID-19. However, its role in the post-infection sequelae, particularly pulmonary fibrosis (PF) remains understudied. Understanding the role of MC in PF following COVID-19 is critical, given the unclear etiology and mechanisms. **Objectives:** this study aimed to investigate whether MC are associated with the development of pulmonary fibrosis in patients who died from SARS-CoV-2 infection, testing the hypothesis that elevated MC expressing CPA3, contribute to lung tissue fibrosis. **Methods:** lung samples from fatal COVID-19 cases were evaluated to assess the number and phenotype of MC using immunohistochemistry and immunofluorescence. Additionally, the presence and extent of fibrosis were analyzed. Statistical analysis was performed to establish correlations between MC density, CPA3 expression, and fibrosis development. **Results:** an increased number of CPA3+ MC was observed in the inflamed lung tissues of severe COVID-19 cases, accompanied by significant changes in pulmonary parenchyma and vasculature. Notably, a higher density of CPA3+ MC correlated positively with the presence of fibrotic tissue. These findings indicate that MC are involved in the inflammatory response during COVID-19 and may contribute to fibrosis through increased recruitment and/or expansion and elevated CPA3 expression. **Conclusion:** these results suggests that the exacerbated inflammatory response in severe COVID-19 drives MC recruitment and CPA3 expression, leading to lung tissue damage and impaired repair mechanisms. This dysregulation may underlie the development of pulmonary fibrosis, highlighting MC as potential therapeutic targets for post-COVID fibrosis.

292. 645. **AHR PLAYS A CRUCIAL ROLE IN MACROPHAGES RESPONSE AGAINST *LEISHMANIA AMAZONENSIS* INFECTION**

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Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*, with a particular emphasis on *L. amazonensis* (La) in Brazil. These pathogens are adapted to survive within the host, making the study of immunometabolic aspects crucial. The aryl hydrocarbon receptor (AhR) plays a central role in regulating macrophage responses, impacting arginine metabolism in these cells. Furthermore, studies show that AhR is important in macrophage responses against trypanosomatids, such as those of the *Leishmania* genus. Therefore, a comprehensive understanding of the role of AhR in immunological regulation and arginine metabolism is necessary to elucidate the interactions between the immune system and the parasite, determining how macrophage responses are shaped against this type of infection. To this end, bone marrow- derived macrophages from AhRfloxLysMcre animals (with myeloid-cells conditional AhR deletion AhRKO) and AhRFlox (control) were cultured and infected with *L. amazonensis*. Gene and protein expression were assessed by qPCR and Western Blot, as well as parasitic load by ImageJ software. A reduction in AhR protein levels and gene expression was observed after 24 hours of infection, indicating that AhR may be a potential target of the parasite's escape mechanisms. Macrophages from AhRKO animals showed a weaker response against *L. amazonensis*, with increased parasitic load. Pretreatment with IFN-gamma increased AhR protein levels, enhancing macrophage inflammatory response through IL-6 secretion, and higher expression of TNF, NOS2, and NOX2; however, this effect is lost in the AhR deletion model. *In vivo*, AhRKO animals exhibited accelerated lesion progression and greater tissue disorganization. These data indicate that AhR plays a crucial role

in machophage response, especially in the early stages of infection.

293. 651. PERIODONTITIS-DERIVED EXTRACELLULAR VESICLES IMPAIRS GLUCOSE HOMEOSTASIS AND INDUCE INFLAMMATION IN INSULIN-TARGET ORGANS

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Background: Periodontitis is an immune inflammatory disease. Epidemiologically, it is related to the occurrence, development, and poor prognosis of type 2 diabetes (T2D). However, the mechanisms underlying this relationship remain unknown. We have postulated that periodontitis-derived extracellular vesicles (Perio-EVs) present in the gingival crevicular fluid (GCF), could modulate glucose metabolism by inducing an inflammatory response and impairing glucose homeostasis. **Objective.** To assess the effect of perio-EVs obtained from the host, or periodontal bacteria outer membrane vesicles (OMVs) on mice glucose metabolism and the expression of inflammatory cytokines in insulin-target organs. **Methods.** Adult male C57BL/6 mice were daily injected intravenously for four weeks with 10^7 Perio-EVs isolated from the GCF of periodontitis-affected patients, with 10^7 and 10^9 OMVs isolated from *Fusobacterium nucleatum (Fn)* cultures, or PBS (control). Weight, glucose tolerance, and insulin sensitivity were assessed at baseline, after 2- and 4-weeks treatment. For this, fasting intraperitoneal (i.p) glucose tolerance test (1g/kg of glucose) and i.p insulin tolerance test (0.75 IU/kg of insulin) were performed. Finally, mice were euthanized, and skeletal muscle and liver were collected to assess IL-1β, IL-6, and TNF-α expression by RT-qPCR**. Results.** Glucose tolerance and insulin sensitivity were similar at baseline across conditions. However, by week 2 and 4, the systemic administration of *Fn*-OMVs induced impaired glucose tolerance and insulin sensitivity ($p<0.05$), with 10^9 *Fn*-OMVs having a greater impact ($p<0.0001$), vs control mice. Also, the administration of Perio-EVs upregulated the expression of IL-6, TNF- α and IL-1β in the liver and skeletal muscle, while *Fn*-OMVs upregulated cytokine expression only in liver. Weight gain remained stable during the 4-week treatment. **Conclusion.** Perio-EVs induced an inflammatory state in insulin-target organs, leading to dysregulation of glucose metabolism in mice. These results support the effect of Perio-EVs as a novel mechanism explaining the well-known association between periodontitis and T2D.

294. 653. INTERACTIONS OF PRIMARY MACROPHAGES, PMNS, AND MDSCS WITH SAGS FROM THE EGC OPERON

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Bacterial superantigens (SAgs) are enterotoxins typically produced by *S. aureus* that trigger an exacerbated immune response, often leading to toxic shock syndrome and other complications. This study investigates how staphylococcal SAgs interact with innate human immune cell cultures. To this end, staphylococcal enterotoxins I (SEI), N (SEN), O (SEO) and U (SEU) were cloned, expressed in *E. coli*, and purified by affinity and molecular exclusion. Macrophages from PBMCs were incubated with SAgs for 1 h or 48 h. Cell death was assessed using Acridine Orange and Ethidium Bromide staining, and supernatants were analyzed for pro-inflammatory cytokines by ELISA. Respiratory burst was measured using dihydrorhodamine 123 (DHR). PMNs were treated with SEN and SEU for 40 min to evaluate respiratory burst activation by flow cytometry using 123 (DHR) staining. Additionally, the effects of SAgs

from the *egc* operon (SEU, SEI, SEO) on Myeloid-Derived Suppressor Cells (MDSCs) were assessed by flow cytometry after a 24-h incubation and staining with an antibody panel. Results showed that macrophages incubated with SAgs exhibited increased cell death by late apoptosis compared to the control ($p < 0.05$). Furthermore, there was a significant release of IL-6, IL-12, and TNF-α ($p < 0.05$), and a tendency towards increased respiratory burst in these cells. Additionally, SAgs significantly increased DHR signal in PMNs, indicating the release of reactive oxygen species ($p < 0.05$). Lastly, a decrease in the percentage of MDSCs was observed following incubation with SEU ($p < 0.05$), SEI, and SEO, including changes in the proportions of the MDSC subpopulations. In conclusion, SEN and SEU activate human macrophages and neutrophils, promoting macrophage death by late apoptosis. Furthermore, only SEU impact MDSCs, which could enhance the effects of SAgs on the immune system.

295. 660. **EFFECT OF SERINE PROTEASES INHIBITION ON SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) MURINE INFECTION**

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) can cause self-limited gastrointestinal infections, bloody diarrhea, and a severe systemic condition known as hemolytic uremic syndrome (HUS). STEC are noninvasive pathogens that colonize the intestine and release Stx, which upon translocation to the bloodstream can trigger the characteristic HUS triad of non-immune hemolytic anemia, thrombocytopenia, and renal injury. Neutrophils are recruited into the gut after infection with STEC, where they can deploy their microbicide battery to kill the pathogen. However, they might also contribute to the inflammatory response accompanying STEC infections, which can impact on the development of HUS. Previously, we determined that neutrophils secrete IL-1β upon challenge with STEC *in vitro* and this secretion can be reduced not only by caspase-1 inhibitors but also by serine proteases inhibitors (SPi). In this work, by employing a murine model of HUS, we aimed to determine whether treatment of mice with a SPi might modulate disease development. To this end, C57BL6 mice were withheld from feeding for 4 h immediately after weaning and then were gavaged with STEC (O157:H7; 10^{10}- 2.5 x 10^{10} colony forming units). SPi or vehicle were administered daily by intraperitoneal injection starting before infection. STEC-infected mice showed clinical signs of HUS such as body weight loss, morbidity, increased plasma urea levels, renal damage, and death by day 5 post-infection. Treatment of STEC-infected mice with SPi significantly reduced mortality as determined by Kaplan-Meier survival analysis ($p<0.05$). Although both groups experimented body-weight loss, it was only significantly different from the sham- group (non-treated-non-infected group) in the STEC-infected vehicle-treated mice ($p=0.005$). A similar result was observed when we analyzed the food intake ($p<0.005$). Altogether, these findings show the potential of SPis to reduce the severity of STEC infections in mice and pave the way for their further pre-clinical exploration as a novel strategy in HUS treatment.

296. 666. **OBESITY AS AN AGGRAVATING FACTOR IN PATIENTS INFECTED WITH COVID-19: INCREASED ANGIOTENSIN II AND INTERLEUKINS**

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With the emergence of COVID-19, several studies have sought to elucidate the association between obesity and elevated Angiotensin II (Ang II) in critically ill patients, although the findings remain conflicting to date. The aim of this study was to analyze the plasma concentrations of Angiotensin II and interleukins in individuals infected with SARS-CoV-2, stratified by Body Mass Index (BMI). Cross-sectional cohort study, with adult patients of both sexes, stratified as Lean, Overweight and Obese. Confirmed diagnosis of COVID-19 by RT-PCR, in the acute phase of infection. The severity of symptoms was classified in hospitalized patients or those under home

monitoring. During the years 2020 and 2022. Statistical analyzes were by GraphPad Prism 8.0. Analysis of variance with Two-Way ANOVA, Tukey's post hoc. Pearson correlation was used, $p < 0.05$. The results showed a significant increase in interleukin-1, in the groups of obese patients, both in mild cases of influenza-like syndrome and in severe cases of Severe Acute Respiratory Syndrome, caused by COVID-19. Interleukin-6 was elevated in the groups with mild and severe obesity, reinforcing the inflammatory role of obesity in clinical worsening. Tumor Necrosis Factor increased, caused by viral infection, while interleukin-10, with anti-inflammatory function, showed reduced levels in obese patients with COVID-19. Plasma levels of Ang II were substantially higher in patients with mild symptoms, but doubled in those with severe COVID-19. A positive correlation was observed between BMI and Ang II levels ($R = 0.6$, $p < 0.001$). This condition is exacerbated by SARS-CoV-2 infection, which competes for Angiotensin-Converting Enzyme 2, further dysregulating the Renin-Angiotensin-Aldosterone System. In conclusion, this study was the first to demonstrate a robust positive relationship between obesity and COVID-19 severity, evidenced by increased plasma levels of Ang II, IL-1β, IL-6, and TNF, with minimal change in IL-10.

297. 685. **IDENTIFYING IMMUNE CELL SUBPOPULATIONS IN HANTAVIRUS CARDIOPULMONARY SYNDROME THROUGH BULK RNA-SEQ DECONVOLUTION**

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Background: Hantavirus Cardiopulmonary Syndrome (HCPS) is a zoonotic disease with a high fatality rate of around 30%, caused by hantaviruses endemic to the Americas. The *andesense* species (ANDV), present in Chile and Argentina, is the only hantavirus capable of human-to-human transmission, giving it pandemic potential. HCPS is marked by severe cardiopulmonary failure, often with hepatic and renal dysfunction. Over 85% of patients rapidly progress to severe HCPS, while some experience mild, flu-like symptoms like fever, headache, and fatigue. The disease's pathophysiology includes vascular leakage from endothelial dysfunction and an exacerbated immune response, leading to a cytokine storm. Understanding immune cell heterogeneity during infection may clarify why some patients develop severe disease, while others do not. **Objectives:** To implement a deconvolution pipeline to analyze bulk RNA-seq data from peripheral blood mononuclear cells (PBMCs), and identify immune cell subpopulations associated with varying HCPS severity. **Methods:** Bulk RNA-seq data were obtained from PBMCs of 36 ANDV-infected patients (8 mild, 28 severe) and sequenced on the Illumina HiSeq 2500 platform, generating 50 million paired-end reads. After quality control, a normalized expression matrix was created, and a deconvolution pipeline was implemented using R (4.4.1) and the granulator package (1.12.0). The pipeline used four PBMC reference profiles and was tested across seven deconvolution methods. Cell proportions were also determined by leukocyte count and flow cytometry. **Results:** We identified transcriptional signatures of 17 distinct PBMC subpopulations, with high correlations between deconvolution methods, demonstrating robust approach. Additionally, the deconvolution results were validated by correlations with clinical data and flow cytometry analysis, reinforcing our findings. **Conclusion:** The deconvolution pipeline successfully revealed cellular heterogeneity among PBMC subgroups in ANDV-infected patients, and the approach was validated by flow cytometry data.

298. **691. INFLUENCE OF POLYMORPHISMS IN THE *PPAR ALPHA* AND *SOAT1* GENES ON LATENT TUBERCULOSIS INFECTION AND LIPID PROFILE OF PRISON STAFF**

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Background: Latent tuberculosis infection (LTBI) associated with factors that promote immunomodulation, such as single nucleotide polymorphisms (SNP) and metabolic changes, may favor the formation of foamy macrophages, cells that do not provide effective protection against the bacillus, contributing to the risk of progression to active disease. **Objectives:** To evaluate the influence of rs 1800206 in the *PPAR alpha* (Peroxisome proliferator activated receptor alpha) and rs 1044925 in the *SOAT1* (Sterol-O-Acyltransferase 1) genes on LTBI and lipid profile of prison staff. **Methods:** Prison staff from a penitentiary in western São Paulo State/Brazil (n=84) was evaluated for: LTBI (QuantiFERON®-TB Gold PLUS test); SNP genotyping by qPCR; lipid profile (total cholesterol, HDL (high-density lipoprotein) and triglyceride) by colorimetry. Statistical analysis was performed using Chi square, Fisher and Mann-Whitney tests ($p<0.05$). **Results:** rs 1800206 CC genotype presented higher HDL levels than CG/GG (p=0.0021); CG/CC higher LDL levels than CC (p=0,0497); LTBI(-) CC higher HDL than LTBI(-) CG/GG (p=0,0006). rs 1044925 AC genotype presented higher triglyceride levels than AA (p=0.0266); LTBI(-) AA higher cholesterol than LTBI(+) AA (p=0.0113); LTBI(-) AA higher cholesterol than LTBI(-) CC (p=0.0198); LTBI(-) AC higher HDL than LTBI(+) AC (p=0.0181); LTBI(-) AA higher LDL than LTBI(+) AA (p=0.0296). **Conclusion:** Studies have shown that low cholesterol levels are a risk factor for the development of active disease and that tuberculosis patients with worse prognosis have low cholesterol and LDL levels, a fact that could be associated with the induction of SOAT1 expression by *Mycobacterium tuberculosis*. It has also been demonstrated that elevated HDL can reduce the inflammatory response against mycobacteria. Thus, we suggest that LTBI(+) individuals with rs 1044925 AA genotype could have higher risk of developing active disease.

299. 696. **CANNABIDIOL MODULATES ARYL HYDROCARBON RECEPTOR SIGNALING, AFFECTING IFN-I AND NF-κB RESPONSES**

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor involved in immune regulation. Our prior studies suggest that AHR signaling limits IFN-I and NF-κB-mediated antiviral responses, thus promoting the replication of viruses such as Zika virus (ZIKV), and dengue virus (DENV). Cannabidiol (CBD), a non-psychoactive cannabinoid from *Cannabis sativa*, exhibits broad-spectrum antiviral activity and partial immunomodulatory effects against ZIKV, as demonstrated in our previous research. In this study, we aimed to investigate CBD's effects on AHR signaling, its impact on IFN-I expression, the NF-κB pathway, and the early stages of ZIKV replication. To achieve this, mRNA expression levels of IFN-beta, AHR, and TDO were measured by RT-PCR, while AHR and NF-κB translocation was quantified via immunofluorescence. NF-κB activation was assessed using a luciferase luminescence assay. Furthermore, IL-6 and IL-8 levels in DENV- and YFV-infected HuH-7 cells were measured by ELISA. Additionally, to study virus entry, viral genome expression was measured after adding CBD at early time points of the replication cycle. Our results revealed that CBD promotes AHR translocation to the nucleus but reduces its activation when combined with a known agonist. RT-PCR data showed that CBD alters AHR and TDO mRNA expression in ZIKV and DENV-infected cells. Furthermore, IFN expression levels in HuH-7 cells peaked at 12 and 48 hours. For NF-κB, a reduction in the nucleus/cytoplasm ratio was observed at 30, 60, and 120 minutes, with CBD decreasing NF-κB transcriptional activation in ZIKV-infected cells. Notably, no induction of IL-6 and IL-8 was detected in HuH-7 cells infected with DENV and YFV. In virus entry assays, CBD significantly affected viral adsorption and internalization, while fusion and genome uncoating were less impacted. Together, these results indicate that CBD affects the AHR signaling pathway, influencing NF-κB and IFN-I responses, and modulates viral adsorption and internalization.

300. 714. **THE SYSTEMIC SPREAD OF PERIODONTAL BACTERIA OUTER MEMBRANE VESICLES DISRUPTS GLUCOSE TOLERANCE, INDUCES SYSTEMIC INFLAMMATION AND ADVERSE PREGNANCY OUTCOMES**

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Background. Periodontitis, a chronic inflammatory gum disease, is implicated in gestational diabetes mellitus (GDM) and adverse pregnancy outcomes (APOs). Nevertheless, the molecular mechanisms that explain this association remain unknown. Recent research has proposed the role of periodontal bacterial (PB)-derived outer membrane vesicles (OMVs) in periodontitis-driven systemic inflammation, a well- known trigger of GDM and APOs. Therefore, we hypothesized that OMVs from periodontitis pathogens *Porphyromonas gingivalis* (*Pg*) and *Fusobacterium nucleatum* (*Fn*) prompt systemic inflammatory responses and alter glucose metabolism leading to APOs.
Objective. To evaluate the effects of PB-OMVs on systemic inflammation, glucose metabolism, and pregnancy outcomes in a periodontitis murine model. **Methods.** PB-OMVs were isolated from *Pg* and *Fn* bacterial cultures by ultracentrifugation. Their biodistribution was assessed *in vivo* using fluorescence imaging following fluorescent dye (DiR) labeling. $1x10^8$ *Pg* or *Fn*-OMVs were administered intravenously (i.v) into the tail vein of periodontitis-affected pregnant mice from pregnancy day E1.5 up to E17.5. Glucose tolerance was measured at baseline and day E15.5 by performing post-fasting intraperitoneal glucose test. On day E18.5, mice were euthanized, and fetuses, liver, pancreas, and spleen were analyzed. Systemic inflammation was evaluated by assessing IL- 1β, IL-6, and TNF-α plasmatic concentration and pancreatic, splenic, and hepatic expression by Luminex immunoassay and RT-qPCR, respectively. **Results.** *Pg*-OMVs severely impaired pregnancy success (<20%) and were excluded from further analyses. DiR-labeled *Fn*-OMVs were predominantly distributed to the liver, spleen, pancreas, kidneys, placentae, and yolk sacs. While baseline glucose tolerance was similar across groups, *Fn*-OMVs significantly impaired glucose tolerance at day E15.5 compared to baseline and control mice. *Fn*-OMVs also increased plasmatic and target organs proinflammatory cytokines levels, affected pregnancy success (~50% vs 80% on controls), and promoted fetal growth restriction reflected in reduced fetal weight and morphometric measurements. **Conclusion.** Systemic administration of PB-OMVs affects fertility, alters glucose metabolism, induces systemic inflammation, and fetal growth restriction. **Funding.** FONDECYT Regular 1211471, ANID CHILE.

301. 720. CALPROTECTIN (S100A8/A9) AS AN INDICATOR OF COVID-19 SEVERITY IN HOSPITALIZED PATIENTS.

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Background: Coronavirus disease 2019 (COVID-19) is a respiratory infectious disease that in severe cases causes acute respiratory distress and exacerbated inflammatory state by inducing neutrophil and macrophage activation. Neutrophils express calprotectin, which magnifies the inflammatory state by releasing proinflammatory cytokines. **Objective:** to associate calprotectin levels with the severity of COVID-19 in hospitalized patients. **Methods:** calprotectin levels were evaluated in 66 patients hospitalized for COVID-19 at the Hospital Civil de Guadalajara Dr. Juan I. Menchaca using the Human Calprotectin ELISA KIT ab267628 (Abcam,

England). In addition, the COVID-GRAM score was calculated and the levels of blood biometry, LDH (lactate dehydrogenase kit; ByoSystems) and IL-6 (high-sensitivity ELISA kit for human IL-6; Invitrogen) were determined. And patients were followed up to observe their progression to invasive mechanical ventilation (IMV) and their outcome. **Results:** significant differences were found in calprotectin levels in patients with IMV (2655 ng/mL) with respect to those who did not require it (1807 ng/mL) and an increase in levels for high risk for COVID-GRAM, types of respiratory supplementation and mortality. Calprotectin levels correlated negatively with lymphocyte percentage (-0.27; p< 0.05) and positively with LHD (0.44; p< 0.05), IL-6 (0.32; p< 0.05), lymphocyte count (0.301; p< 0.05) and neutrophils (- 0.317; p< 0.05). **Conclusion:** Calprotectin levels could be an indicator of COVID-19 severity, as elevated levels were found in patients with need for IMV, high risk on the COVID-GRAM scale and higher mortality, which supports its usefulness in management of patients hospitalized for COVID-19.

302. 729. **CHARACTERIZATION OF EXOSOME-DERIVED MICRORNAS IN YOUNG ADULTS WITH SEVERE DENGUE AND THE IMPLICATIONS OF MIR-15A-5P IN INFLAMMATION AND DISEASE SEVERITY**

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Dengue, a viral infection transmitted by Aedes aegypti/albopictus mosquitoes, has surged dramatically over the last five decades. The 2023-2024 period is regarded as the worst dengue season in the history of the Americas, according to PAHO. Emerging evidence indicates that microRNAs (miRNAs) within exosomes significantly contribute to the pathogenesis of various arboviruses, yet their role in dengue remains unclear. This study aimed to elucidate the immunological role of exosomal inflammatory miRNAs in patients with dengue hemorrhagic fever (DHF) to identify molecular signatures for diagnosing severe cases and improving hospitalized patient management. We conducted a prospective study with young adult patients diagnosed with DHF (n=4) and dengue fever (DF) (n=4), enrolled within 24 to 48 hours of admission. We measured serum cytokines and performed miRNA array analysis on exosomes using a qPCR-array platform. Advanced bioinformatics analyses, including gene ontology enrichment and weighted gene co-expression network analysis (WGCNA), were applied to miRNA target genes (Ethics Committee Approval: 24-11-2020) Our results revealed that serum levels of IL-12, TNF-α, and IL-6 were significantly elevated in DHF patients compared to those with DF, accompanied by increased leukocyte and monocyte counts. Both groups showed mild neutropenia and thrombocytopenia, with more pronounced reductions in DHF. Six miRNAs were upregulated in DHF patients, demonstrating transcriptional differences between groups through PCA, volcano plots, and heatmaps. An interactive miRNA-mRNA network identified target genes involved in DNA damage, apoptosis, and inflammatory regulation. Notably, miR-15a-5p exhibited a strong positive correlation with IL-12, IL-8, IL-10, IL-6 levels, and leukocyte and monocyte populations, while being negatively correlated with platelet counts. In conclusion, our preliminary data suggest that miR-15a-5p plays a crucial role in modulating immune responses in DHF, potentially contributing to platelet and immune cell apoptosis, highlighting its importance in disease severity.

INFLAMMATION

303. 027. **IDENTIFICATION AND CHARACTERIZATION OF NEW DEFENSE PEPTIDES WITH ANTI-INFLAMMATORY ACTIVITY ISOLATED FROM THE SKIN OF AMBYSTOMA MEXICANUM**

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Among the new therapeutic strategies to control chronic inflammation are host defense peptides (HDPs), which in addition to present antimicrobial activity, usually have an anti- inflammatory effect. This double activity allows treating inflammatory diseases without compromising the immune response against infectious agents by modulating

local (microenvironment) and systemic immunity processes, encompassing various mechanisms of regulation of the immune response such as chemotaxis, phagocytosis, apoptosis, cell proliferation and production of cytokines, chemokines and reactive oxygen species. HDPs are peptides composed of 12 to 50 amino acids, most of them structured in an alpha helix, and with cationic and amphipathic properties. They usually induce the death of microorganisms by interacting with their membrane, which make them less susceptible to bacterial resistance. Among amphibians, although skin frog HDPs with immunomodulatory properties have been largely identified and characterized, very little is known about Mexican axolotl (*Ambystoma mexicanum*) HDPs. Our research group decided to identify and characterize HDPs from skin secretions of the Mexican axolotl with antimicrobial activity and/or anti-inflammatory effect. Axolotl secretions were obtained from individuals from 4 different environments through a gentle massage without harming or sacrificing the animal (as it is an endemic species of the lacustrine area of Mexico City in the critically endangered category of extinction). Subsequently, the secretions were fractionated by HPLC, and fractions were selected for analysis by mass spectrometry and characterization of the anti-inflammatory capacity through functional assays with human leukocytes (inhibition of migration or proliferation, immunosuppressor effect and cytokine profile) and antimicrobial activity. Statistical analysis was performed using a Shapiro-Wilk normality test followed by an ANOVA test. For the first time, peptides produced by the skin of the endemic Mexican axolotl were identified and at least one of them could be a candidate for treatment of inflammatory diseases.

304. 081. **INMUNE PROFILE OF IN VITRO MONOCYTE-DERIVED MACROPHAGES REMAINS LARGELY UNALTERED IN SPITE OF EARLY EXPOSURE TO PLATELETS**

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Platelets are increasingly recognized as modulators of the immune system. Particularly, they are known to interact with monocytes and modulate their differentiation to macrophages. Previously, we have shown that exposure to prostaglandin E2, a major product of activated platelets, induced a pro-resolutive profile in macrophages. Here, we aim to evaluate the effect of platelet-monocyte interaction on the immune profile of monocyte-derived macrophages. Human monocytes and platelets were isolated from peripheral blood of healthy blood donors. Platelets were added to purified monocytes for 1 or 24 hours, washed, and then monocytes were differentiated to macrophages by culture during 7 days in the presence of M-CSF (50 ng/ml). Initial ratio of platelet to monocyte ranged between 3:1 to 45:1. Activation state of platelets was assessed by expression of marker CD62p before their addition to the cultures. Macrophage phenotype was analyzed by flow cytometry and their efferocytic capacity was quantified by incorporation of CFSE-stained apoptotic Jurkat cells. Flow cytometry analysis of HLA-DR/CD61 double-positive events revealed that addition of platelets led to increased numbers of monocyte-platelet aggregates (2.8% of monocytes in control cultures to 16.8% with platelet:-monocyte ratio of 45:1, n=2). Platelets could be found adhered to macrophages even at day 6 of culture (10.4% in control cultures to 20.5% with platelet:monocyte ratio of 45:1, n=3). Addition of platelets did not affect monocyte viability after 24 hours, according to annexin V / propidium iodide staining (n =5). Exposure of monocytes to platelets for 24 hours did not modulate phenotypic markers CD36 and CD206 (n=5). Exposure of monocytes to platelets for either 1 or 24 hours did not modulate efferocytosis capacity of macrophages (n=5). Our results suggest that early addition of platelets to monocyte cultures in a model of monocyte-derived macrophages with M-CSF does not have substantial effects on the immune profile of macrophages.

305. 095. **LYMPHOCYTE RESPONSE IN WALKER 256 TUMOR PROGRESSION: COMPARATIVE STUDY BETWEEN WISTAR RATS AND NON-OBESE TYPE 2 DIABETIC GOTO-KAKIZAKI RATS**

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Obesity is a known risk factor for insulin resistance, which can lead to the development of type 2 diabetes mellitus and, consequently, promote tumor progression. This connection is largely due to chronic inflammation creating tissue microenvironments conducive to tumor development. However, the impact of insulin resistance on tumor behavior in the absence of obesity remains unclear. The aim of this study was to evaluate the lymphocyte profile during the progression of the Walker 256 tumor in Wistar rats compared to Goto-Kakizaki (GK) rats, which serve as a model for non-obese type 2 diabetes mellitus. The Walker 256 tumor cells were inoculated subcutaneously into the right flank of the animals at a concentration of 2x10□ cells. The study involved 10 eight-week-old GK rats and 10 Wistar rats. Following euthanasia, mesenteric lymph nodes and spleens were collected, and lymphocytes were isolated for gene expression analysis related to lymphocyte differentiation. Our results indicate that GK rats exhibit insulin resistance and have a higher tumor weight-to-body weight ratio compared to Wistar rats. Additionally, lymphocytes from GK rats with tumors secrete more IL-22 and less IL-10 when stimulated with Concanavalin A. These lymphocytes also show higher expression of ROR-gamma and lower expression of GATA-3 and Foxp3 compared to those from Wistar rats. These findings suggest that lymphocytes from GK rats demonstrate enhanced inflammatory activity, potentially altering the tumor microenvironment in a way that promotes tumor progression.

306. 098. **ACTIVATION OF MAST CELLS BY SNAKE VENOM TOXINS**

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This study examined the inflammatory response triggered by the venoms of *Bothrops jararaca* (Bj), *Bothrops atrox* (Ba), and *Bitis gabonica* (Bg) snakes, focusing on mast cell. Mast cells release inflammatory mediators such as histamine, cytokines and the mast cell-specific enzyme called Mast Cell Protease-1 (MCPT-1), which is a plasma marker of histamine release after its activation. The aim was to examine mast cells' role in envenomation and measure histamine and cytokine release at two venom concentrations (30% and 50% of the LD50). Results showed that all venoms induced mast cell degranulation with varying intensities. At 30% of the LD50, Bg venom caused the highest number of degranulated mast cells and in addition, together with the Ba group, it was the group with the highest number of cytokines released. Increasing the concentration to 50% of the LD50 significantly increased total mast cells in all venom-treated groups compared to the PBS control, suggesting enhanced recruitment of these cells to the envenomed site. Cytokine analysis revealed significant increases in the pro-inflammatory cytokine TNF-α and the anti- inflammatory cytokine IL-10 in the Bg-treated group. The Ba group also showed a prominent inflammatory response, with significant elevations in IL-6 and the chemokine MCP-1, indicating intense inflammation and cell recruitment. The cytokine IFN-γ was elevated in the Ba group compared to Bj and in the Bg group compared to the control, suggesting a complex interaction between the venoms and the immune system. The study shows that sublethal venom doses activate mast cells, releasing histamine and other inflammatory mediators. Bothrops and Bitis venoms cause significant mast cell degranulation, crucial in envenomation. Ba venom induces a stronger inflammatory response, while Bg venom also triggers significant mediator release. These findings aid in developing therapies and understanding the immune response in snake envenomation.

307. 103. **INFLUENCE OF BODY COMPOSITION ON POSTPRANDIAL GLUCOSE AND TRIGLYCERIDE PLASMA LEVELS IN FEMALES**

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Physiological processes involved in food digestion and metabolic adaptations to the availability of energy substrates are observed in the postprandial period. Understanding the metabolic and inflammatory changes that occur during the postprandial period is crucial for uncovering the rela-

tionship between these changes and the development of chronic diseases. The aim of the study was to evaluate the relationship between body composition and plasma glucose and triglyceride concentrations after ingestion of a high-glycemic and high-fat meal in women. Seventy-seven women participated in the study. After a 10-hour fast, measurements of body weight, height, waist and hip circumferences, and body composition were recorded. The test meal containing 75 g of glucose, 30 g of palm oil, 30 g of soybean oil and 20 g of micellar casein was offered at 8:00 am. Capillary blood samples were collected at -10, 0, 30, 60, 120, 180, 240 and 300 minutes after meal ingestion. The participants had an average age of 33.88 ± 1.61 years, 33.99 ± 0.98 body fat, 0.76 ± 0.006 waist-to-hip ratio (WHR) and 25.84 ± 0.57 body mass index (BMI). We observed a 23% increase in blood glucose and a 28% increase in plasma triglycerides compared to fasting levels. When dividing the participants into groups according to age, BMI and fat percentage, it is observed that the kinetics of plasma glucose is influenced by the 3 parameters. Volunteers with greater age or BMI >30 have a higher glycemic peak compared to the other groups. Volunteers with a lower body fat percentage and younger age exhibited lower fasting blood glucose levels and a smaller glycemic peak compared to those with a higher body fat percentage or older age. These data confirm previous findings and demonstrate the association between postprandial glycemic dysregulation and factors such as aging, BMI, and body fat percentage.

308. 105. **IDENTIFICATION OF INTERLEUKIN-6 AND ITS RECEPTORS IN THE AORTIC BARORECEPTOR AFFERENTS OF RATS SUBJECTED TO SYSTEMIC INFLAMMATION**

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Background: Recent evidence has suggested that baroreceptors, known for controlling blood pressure, may also have a role in modulating the immune system. We propose that aortic baroreceptor afferents express cytokine receptors to communicate reflexly with the immune system. **Objective:** We aimed to investigate the presence of interleukin (IL)-6 and IL-6 receptors (IL-6R) in aortic baroreceptor afferents from endotoxemic rats. **Methods:** Lipopolysaccharide (LPS; 1.5 mg/kg, i.v.) was used to induce systemic inflammation. The aortic arch (AA), aortic depressor nerve (ADN), and nodose ganglion (NG) were collected from male Sprague-Dawley rats (7-8 weeks old) in different groups: before (Basal) and after the administration of saline (30 min later) or LPS (30, 60, 90 and 120 min later). Gene expression and total protein content were evaluated by RT-qPCR and Western Blot, respectively. Data were analyzed using One-Way ANOVA followed by Tukey's test. **Results:** All the tissues evaluated expressed IL-6 and IL-6R in health control animals (Basal group). IL-6 gene expression was upregulated 120 min after LPS administration in ADN (6.8 ± 0.9 fold-change; $p = 0.0038$), NG (422 ± 42 fold-change; $p < 0.0001$), and AA (9794 ± 2748 fold-change; $p = 0.0009$), suggesting the activation of this cytokine in the baroreceptor afferents during the systemic inflammation. In addition, total protein content of IL-6R also increased 120 min after LPS in AA (0.58 ± 0.24 *vs* 2.31 ± 0.58 IL-6R/beta-actin; $p = 0.0123$). **Conclusion:** These results add a new function to ADN, which until now was known only to have mechanoreceptors related to the detection of blood pressure. Our novel findings, suggest the identification of an afferent system that mediates reflexes evoked by pro-inflammatory cytokines, which participate in modulation of the inflammatory response via baroreceptor afferents. **Funding sources:** FAPESP (2021/03764-8; 2021/05554-0; 2022/13361-0; 2020/06043-7), CAPES (88881.823812/2023-01) and CRID (2013/08216-2).

309. 126. **CITRAL, A MONOTERPENE THAT PROTECTS AGAINST THE INTESTINAL AND SYSTEMIC DAMAGE CAUSED BY**

HIGH-FAT DIET AND LIPOPOLYSACCHARIDE IN MICE

Maycon Tavares Emílio-Silva[1], Vinicius Peixoto Rodrigues[1], Antonio Jesús Ruiz-Malagon[2], Mariana Moraes Fioravanti[1], Matheus Naia Fioretto[1], Rie Ohara[1], Gabriela Bueno[1], Felipe Lima Dario[1], Luis Antonio Justulin Jr[1], Alba Rodríguez-Nogales[2], Julio Gálvez[2], Lúcia Regina Machado da Rocha[1], Clelia Akiko Hiruma-Lima[1]

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Background: Obesity is directly linked to an imbalance in the gut microenvironment and fat accumulation in adipose tissue caused by an unbalanced diet. Overnutrition increases intestinal permeability, facilitating greater absorption of nutrients, resulting in metabolic and inflammatory alterations. **Objectives:** We investigated the effects of citral (CT) on systemic and tissue damage associated with lipopolysaccharide (LPS) and a high-fat diet (HFD) in *in vitro* and *in vivo* models. **Methods:** Adult Male C57BL/6J mice (n=10 per group) were fed a standard diet and HFD for 17 weeks, with daily oral treatment with CT (25, 100, or 300 mg/kg) and 1% Tween 80 (10 mL/kg) (Process No. 6702310820). Body mass gain and caloric intake were assessed twice weekly. Histological parameters, lipid profile, adipose index, systemic cytokine levels, and gene expression in the colon were determined. Murine rectal carcinoma (CMT-93) cells against LPS (10 µg/mL) stimulus for 72h were used to assess the protein expression. One-way and two-way ANOVA followed by Tukey's *post-hoc* test was used for statistical analysis ($p<0.05$). **Results:** Treatment with CT (300 mg/kg) protected against an increase in body mass gain compared to HFD mice. This response was due to the anti-inflammatory effect of CT in the increase in NLRP3 inflammasome gene expression, which also reduced the cellular infiltrate in the colon caused by HFD. The monoterpene also caused a significant reduction in the adiposity index and serum levels of total and LDL cholesterol ($p<0.05$). CT treatment caused a significant decrease in the serum levels of IL-1 and TNF-α when compared with the SD group ($p<0.05$). In addition, CT reduced the expression of iNOS and maintained ZO-1 levels in LPS-stimulated CMT-93 cells ($p<0.05$). **Conclusion:** CT exerts antiobesogenic, anti-inflammatory, and antihyperlipidemic actions. These effects shown promising preclinical results as a protective agent against the detrimental effect of HFD and LPS in mice.

310. 135. **TNF RECEPTORS POLARIZATION TO INFLAMMATORY PROFILE IN CD4 SUBPOPULATIONS OF PREGNANT WOMEN WITH PREECLAMPSIA**

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Background: Preeclampsia is a pregnancy-specific syndrome characterized by intense systemic inflammatory response and imbalance between Th17 and regulatory T (Treg) cells. This imbalance is associated with elevated production of pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin-17 (IL-17) and deficiency in the anti-inflammatory cytokine IL-10. TNF can exert different functional effects on CD4+ T cell subpopulations through interaction with its receptors TNFR1 and TNFR2. **Objective:** To investigate the expression of TNFR1 and TNFR2 by Th17 and Treg cells in preeclamptic women and their association with the intracellular concentrations of TNF and IL-17. **Methods:** The study included 20 preeclamptic women and 20 normotensive controls matched by gestational age. Flow cytometry was used to assess the expression of TNFR1 and TNFR2, the transcription factors RORγt (Th17), FoxP3 (Treg), and the intracellular cytokines TNF and IL-17 in CD4+ T cells from peripheral blood. Plasma concentrations of TNF, IL-17, and IL-10 of preeclamptic and normotensive pregnant groups were evaluated by ELISA. Data were analyzed using non-parametric tests. **Results:** Compared to the normotensive group, preeclamptic women exhibited significantly higher plasma levels of TNF and IL-17 and lower levels of IL-10. The percentage of CD4+ T lymphocytes co-expressing TNFR1 and the transcription factors RORγt (Th17) and FoxP3 (Treg) was significantly higher, while TNFR2 expression was significantly lower in the preeclamptic group. Intracellular TNF expression was higher in CD4

T cells expressing TNFR1 and TNFR2 from preeclamptic women, while IL-17A expression was higher in TNFR1-positive cells and lower in TNFR2-positive cells. **Conclusion:** The results confirm that preeclamptic women presented an imbalance in Th17/Treg lymphocytes together with increased TNFR1 expression and decreased TNFR2 expression by these cells. This suggests that high circulating levels of TNF play a role in the polarization of these cells towards an inflammatory profile and impair the regulatory function of Treg cells in preeclampsia.

311. 139. **DOWNREGULATION OF NLRP3 INFLAMMASOME AND PYROPTOSIS BY MgSO4 IN MONOCYTES FROM SEVERE PREECLAMPSIA**

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Background: Hypertensive disorders of pregnancy are one of the leading causes of maternal and perinatal mortality worldwide. Preeclampsia (PE) is a multisystemic condition characterized by intense activation of innate immune cell components, such as the NLRP3 inflammasome, which is responsible for the release of interleukin-1 (IL-1beta) and IL-18. PE can progress to critical situations, including hypertensive crisis, HELLP syndrome, and eclampsia. Eclampsia is defined by the occurrence of generalized tonic-clonic seizures and is one of the most severe complications of the disease. Evidence in the literature shows that magnesium sulfate (MgSO4) is the treatment choice for the prevention and management of eclampsia, in addition to having anti-inflammatory effects. **Objectives:** To evaluate the anti-inflammatory effect of intravenous administration of MgSO4 on the NLRP3 inflammasome and pyroptosis expression in monocytes of pregnant women diagnosed with severe preeclampsia. **Methods:** Blood from 16 pregnant women with severe PE was collected before and at 12 and 24 hours of MgSO4 treatment. Flow cytometry assessed the protein expression of NLRP3, caspase-1, gasdermin D (GSDMD), and intracellular cytokines TNF, IL-1beta, IL-18, and IL- 10 in monocytes cultured for 4 hours with or without monensin and brefeldin A. Results were analyzed using non-parametric tests with a significance level of 5%. **Results:** The results showed that the expression of NLRP3, caspase-1, GSDMD, IL-1beta, and IL-18 were significantly higher in monocytes before MgSO4 treatment. However, the administration of MgSO4 induced a decrease in these protein expressions at 12 and 24 hours. IL-10 exhibited lower expression in monocytes before MgSO4 treatment, and increased at 12 and 24 hours following MgSO4 administration. ***Conclusion:*** MgSO4 treatment determined an early and efficient downregulatory effect on innate immunity response by decreasing NLRP3 inflammasome and pyroptosis activation while upregulating the expression of IL-10 in monocytes from severe preeclampsia

312. 140. **IMMUNOMODULATORY EFFECTS OF BRAZILIAN GREEN PROPOLIS EXTRACT ON MONOCYTE ACTIVATION IN PREGNANT WOMEN WITH PREECLAMPSIA**

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Background: Preeclampsia (PE) is an important pregnancy-specific syndrome characterized by an exacerbated inflammatory response, which prompted us to investigate new agents that could modulate this inflammatory state. Propolis, is a honeybee product, displaying various pharmacological properties, particularly immunomodulatory and anti- inflammatory effects. **Objective**: The present study aimed to evaluate the immunomodulatory effect of a Brazilian green propolis extract on signaling pathways and intracellular cytokines in monocytes from pregnant women with PE. **Methods:** Monocytes were isolated from twenty pregnant women with PE and twenty normotensive (NT) pregnant women matched for gestational age. Cells were cultured with or without propolis, brefeldin A, and monensin for 30 minutes or 4 hours. The expression of ERK1/2, p65NF-κB, CD192 receptor, and intracellular cytokines (IL-1β, IL-6, IL-12, IL-10, and TNF) were assessed by flow cytometry. Statistical analysis

was performed using the Kruskal-Wallis test with a significance level at 5%. **Results:** Monocytes from pregnant women with PE showed significantly higher endogenous expression of ERK1/2, p65NF-κB, CD192 receptor, and inflammatory cytokines, while IL-10 expression was significantly lower compared to NT group. The treatment of monocytes with propolis reduced significantly the percentage of cells expressing the transcription factors, CD192 receptor, and inflammatory cytokines and, notably increased the percentage of monocytes expressing IL-10 in PE group. **Conclusions:** The results demonstrated that the Brazilian green propolis extract exhibited an immunomodulatory effect, by attenuating the activation of intracellular pathways and inflammatory cytokines while upregulating the IL-10 expression in monocytes. These findings suggest that propolis may be a potential adjunctive therapy in the management of PE.

313. 141. **ROLE OF CD44 RECEPTOR IN Th1/Th17/Treg SUBPOPULATION ACTIVATION IN PREGNANT WOMEN WITH PREECLAMPSIA MEDIATED BY HYALURONAN**

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Background: Pregnant women with preeclampsia (PE) show an intensified immune response, marked by elevated plasma levels of damage-associated molecular patterns (DAMPs) like hyaluronan (HA). When HA is degraded into low molecular weight fragments (lmw-HA), it triggers an inflammatory response, activating immune cells and increasing pro-inflammatory cytokines, such as tumor necrosis factor (TNF). **Objectives:** This study aims to analyze CD44 expression on $CD4^+$ T lymphocytes in pregnant women with PE, before and after treatment with high molecular weight hyaluronan (hmw-HA) or low molecular weight hyaluronan (lmw-HA), to better understand the effect of treatments in the activation of Th1, Th17, and Treg lymphocyte subpopulations in PE. **Methods:** The study included 20 preeclamptic and 20 normotensive pregnant women, matched by gestational age. Plasma samples were analyzed for TNF, IL-10, HA, and soluble CD44 (sCD44) concentrations using ELISA. Flow cytometry assessed CD44 receptor expression, intracellular transcription factors T-bet (Th1), RORγt (Th17), Foxp3 (Treg), and intracellular cytokines TNF, IL-17, and IL-10. These analyses were conducted before and after hmw-HA and lmw-HA cell culture for 30min or 4h. Results were analyzed using Kruskal-Wallis test with a significance level at 5%. **Results:** Women with PE had significantly higher plasma concentrations of HA and TNF, but lower levels of sCD44 and IL-10 compared to normotensive group. The proportion of CD44-expressing cells were increased in T-bet, RORγt, TNF, and IL-17 T $CD4^+$ lymphocytes. Culture with lmw-HA increased T-bet and RORγt while reduced FoxP3 in normotensive women. Conversely, hmw-HA decreased RORγt, IL-17, and TNF, and increased FoxP3 and IL-10 in PE patients. **Conclusion:** The findings highlight the significant modulatory role of hmw-HA in restoring Th17/Treg balance, reducing inflammation, and enhancing the anti-inflammatory response in PE.

314. 142. **DOWNREGULATION OF M2-LIKE MONOCYTES IS ASSOCIATED WITH DECREASED EXPRESSION OF TNFR2 IN PREGNANT WOMEN WITH PREECLAMPSIA**

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Background: Monocytes are important cells of innate immunity whose activation process is closely associated with the development of preeclampsia (PE) a specific syndrome of pregnancy characterized by an intense inflammatory response. These activated cells produce high levels of the pro-inflammatory cytokine tumor necrosis factor (TNF) that may interact with TNF receptors (TNFR1 and TNFR2) inducing pro- and anti-inflammatory effects, respectively. The predominant TNF interaction with TNFR2 plays an anti-inflammatory role by increasing the release of IL-10 while TNF binding with TNFR1 induces an increase in pro-inflammatory cytokines production. **Objective**: The present study aimed to evaluate whether the expression of TNFR2 might

be associated with M2-like monocyte polarization in pregnant women with PE. **Methods:** Monocytes obtained from seven preeclamptic pregnant women and seven normotensive (NT) pregnant women were evaluated for the endogenous expression of TNFR2. In addition, the endogenous expression of TLR4, CD64 (M1-like) receptors, and CD163, CD206 (M2-like) receptors were assessed by flow cytometry. Statistical analysis was performed using the Mann-Whitney test with a significance level of 5%. **Results:** The median fluorescence intensity (MFI) of monocytes expressing TNFR2 and CD163 were significantly lower in preeclamptic women than in the NT group. Similarly, the monocytes from PE showed a significantly reduced percentage of CD163 and CD206. On the other hand, the MFI of TLR4 and CD64 were significantly higher in the PE group when compared to NT pregnant women. **Conclusions:** The results obtained suggest that the systemic inflammation present in women with PE is associated with decreased expression of TNFR2 and downregulation of M2-like monocytes.

315. 145. **DOWNREGULATED EXPRESSION OF IMMUNE CHECKPOINTS AND TNFR2 IN REGULATORY T CELLS OF PREGNANT WOMEN WITH PREECLAMPSIA**

Patricia Braga da Silva[1], Mariana Romao-Veiga[2], Vanessa Rocha Ribeiro-Vasques[2], Gabriela de Oliveira Franco[1], Larissa Ragozo Cardoso de Oliveira[2], Jose Carlos Peracoli[1], Maria Terezinha Peracoli[2]

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Background: Preeclampsia (PE) is a pregnancy-specific syndrome characterized by an adaptive immune response that is skewed towards an inflammatory profile to the detriment of the regulatory profile mediated by regulatory T cells (Treg). Molecules called immune checkpoints inhibitors and tumor necrosis factor receptor 2 (TNFR2) are expressed mainly in Treg cells and play a crucial role in the balance between pro- and anti-inflammatory signals that occur at the maternal-fetal interface to ensure maternal tolerance and the success of the pregnancy. **Objective:** This study aimed to evaluate the expression of the immune checkpoint inhibitors CTLA-4, PD-1, and GITR, and tumor necrosis factor receptor 2 (TNFR2) by regulatory T cells from pregnant women with PE. **Method:** Fourteen pregnant women were studied, 7 with PE and 7 normotensive (NT) pregnant women, matched by gestational age. CD4+ T cells from peripheral blood were isolated to analyze the expression of CTLA-4, PD-1, 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, preeclamptic women exhibited a significantly lower percentage of Treg cells. Similarly, the median fluorescence intensity (MFI) of CTLA-4, PD-1, GITR, and TNFR2 expression in Treg cells was lower in the preeclamptic group. **Conclusion:** The results show an association between TNFR2 and the immune checkpoint inhibitors and confirm the impairment of Treg cells in preeclampsia.

316. 149. **MODULATION OF INFLAMMATION INDICATORS BY NATURAL ANTIOXIDANTS IN MONOCYTES- DERIVED MACROPHAGES**

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Obesity is characterized by pro-inflammatory state with macrophage infiltration in adipose tissue. We showed that antioxidant extracts (EXTs) obtained from *Baccharis articulata* (C), *Peumus boldus* (B), and *Verbena bonaerensis* (V) inhibited almost 30% triglyceride content on *in vitro* mature adipocytes. We aim to study immunomodulatory activity of these EXTs on monocyte-derived macrophages (Mφ). EXTs were obtained from leaves at 37°C for 1-h. THP-1 monocytes were induced to differentiate to Mφ with PMA for 72-h. We performed 24-h treatments with the EXTs on Mφ and determined proliferation rate by MTT assay (arbitrary units), cellular activation through nitrite (Nit) production (μM) by Griess method, and secretion of IL-1β and IL-10 by ELISA (ρg/mL), using antioxidant gallic acid (GAL) as control. Statistical analyses were performed using one-way analysis of variance followed by Dunnett's multiple comparisons test against non-treated cells (Mφcc), considered as 100%. EXTs were anti-proliferative, $p<0.01$ (100.00 ± 2.81 [Mφcc], 71.21 ± 0.39 [Mφ+C], 74.80 ± 1.84 [Mφ+B], 83.43

± 3.19 [Mφ+V]). All of them decreased Nit level, only B showed a significant difference p<0.05 (100.00 ± 30.53 [Mφcc], 67.37 ± 6.86 [Mφ+C], 12.13 ± 1.58[Mφ+B], 78.65 ± 6.06 [Mφ+V]). PMA increased pro-inflammatory level IL-1β by 27% in Mφcc compared to monocytes; EXTs also increased it, p<0.05 (100.00 ± 14.51 [Mφcc], 357.40 ± 118.20 [Mφ+C], 509.20 ± 85.86 [Mφ+B], 417.60 ± 19.91 [Mφ+V]), GAL significantly reduced it by 37%. Only C increased level of anti-inflammatory IL-10, p<0.01 (100.00 ± 10.10 [Mφcc], 257.20 ± 10.11 [Mφ+C]). EXTs were able to modulate negatively cellular proliferation and activation. PMA induced Mφ phenotype M1 increasing IL-1β secretion, while GAL reverted this effect, EXTs couldn´t decrease it. C showed a possible switch on Mφ phenotype from M1 to M2 inducing anti-inflammatory cytokine. We suggested that natural antioxidants could have a role in inflammation process modulation.

317. 162. **CYTOKINE PRODUCTION BY T LYMPHOCYTES OF TITANIUM HYPERSENSITIVE PATIENTS AND HEALTHY DONORS AFTER STIMULATION BY ADVANCED TITANIUM-BASED MATERIALS**

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Main goals were: to prepare innovative titanium surfaces with intermixed thin nanolayers doped with silver; to determine the effects of modified titanium surfaces on the proliferation of T lymphocytes of titanium hypersensitive patients and of healthy donors; to establish the production of 10 cytokines after stimulation with titanium and silver ions, sample materials and infusions from these samples. The MELISA® test was used to establish the reaction of patients' T lymphocytes to titanium and silver ions, non-treated titanium surface, Ag doped titanium surface and infusions prepared from such samples. The proliferation of T lymphocytes from 16 healthy donors and from 19 titanium hypersensitive patients was evaluated. A set of pro-inflammatory and anti-inflammatory cytokines (IL-1 alpha, IL-1 beta, IL-4, IL-6, IL-8, IL-10, IL-13, IFN gamma, TNF alpha, MCP-1) was determined by multiplex analysis. Positive reaction of patients' T lymphocytes to TiO_2 and/or $TiCl_3$ was determined in all 19 patients and to silver in 11 patients. Weakly positive reaction of patients' T lymphocytes to 4 samples were determined in 1 patient. Reactivity to all other tested substances in this group were negative. Weakly positive reaction of healthy donor´s T lymphocytes to TiO_2 was determined in 1 patient and to silver in 2 patients. Reactivity to all other substances in this group was negative. Statistically significant increased production (P≤0.05) of IL-1 alpha, IL-1 beta, IL-4, IL-6, IL-8, IL-10, IL-13, TNF alpha and MCP-1cytokines was found in patient group. Hypersensitive patients' T lymphocytes testing is a promising in vitro method for preclinical testing of newly developed implant materials.

318. 168. **THE POLYMORPHISM A>G OF INTERLEUKIN-6 (RS1800797) IS ASSOCIATED WITH ANTHROPOMETRIC MARKERS IN MEXICAN MESTIZO PEOPLE LIVING WITH OBESITY.**

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Background. Obesity is a serious worldwide pathology involved in the development of comorbidities as diabetes, cardiovascular diseases, cancer, etc. Chronic proinflammation in people living with obesity, has been identified as a pivotal factor triggering comorbidities. Genetic characteristics are relevant factors related to obesity and comorbidities. Interleukin-6 gen has been associated with several metabolic diseases. **Objectives**. To identify the association of the SNP A>G of Interleukin-6 gen (rs1800797) in Mexican mestizo people living with Obesity (OB) and Normal

Weight (NW). **Methods.** Anthropometric, clinic and biochemical markers were evaluated in all the participants, and all of them signed informed consent. DNA was obtained from peripheral sample from OB group (n=135) and NW group (n=136). The genotyped was done by PCR-RQ method using TaqMan probes. Chi squared was used to analyze the genotype frequency and t-student or Wilcoxon were used to compare clinic, anthropometric and biochemistry values between groups. A $p<0.05$ was used as a statistical difference. **Results.** Body Mass Index in OB group was: =39.59+/-9.3, and =21.74+/-1.54 in NW group ($p<0.05$). There was no statistically difference ($Xi^2=0.62$, $p<0.42$; O/R=0.88; I.C.95% 0.35-1.75) in the frequency (0.44) of OB carriers (A-G + G-G) versus frequency (0.55) of NW carriers. When were compared OB carriers versus OB wild type (A-A), no differences in clinical and biochemical values were observed. However, waist circumference: 113 cm vs 99 cm; waist/hip index: 0.91 vs 0.84 and visceral abdominal area: 182 cm^2 vs 157 cm^2, respectively, were statistically higher ($p<0.05$) in OB carriers. **Conclusion**. Mexican mestizo people living with obesity from central Mexico, not showed statistically differences in the frequency of genotype of SNP rs1800797 versus NW. OB carriers of G allele, showed major values of visceral fat, waist circumference and waist/hip ratio, relevant markers associated to development of comorbidities of obesity as diabetes, dyslipidemia and cardio-cerebrovascular diseases.

319. 181. **EXTRACELLULAR VESICLES FROM NEUTROPHILS PROMOTE PROINFLAMMATORY EFFECTS ON PERIPHERAL BLOOD MONONUCLEAR CELLS THROUGH NF-KB SIGNALING PATHWAY**

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Background: Extracellular vesicles (EVs) are small particles limited by lipidic bilayer membrane, secreted by cells to the extracellular environment, where they participate in cellular communication, regulating physiological functions. Neutrophil-derived EVs act as mediators of proinflammatory and anti- inflammatory responses. However, the effects of the interaction of neutrophil- derived EVs with peripheral blood mononuclear cells (PBMCs) are not completely clear. **Objectives:** Here, we investigated whether neutrophil EVs promote immunomodulatory activity in human PBMCs. **Methods:** Neutrophils and PBMCs were obtained from buffy coats of healthy donors by density gradients, and EVs were isolated from neutrophil culture supernatant by ultracentrifugation. PBMCs were stimulated with EVs at concentrations between 6.25-100µg/mL and, after 24h, the cytokine levels were measured in the culture supernatants by ELISA. For analysis of NF-kB activation, PBMCs were treated with 50µg/mL of EVs, and NF-kB p65 phosphorylation was measured by ELISA. In some assays, EV-exposed PBMCs were pre-treated with an NF-kB inhibitor, followed by measurement of cytokine levels after 24 hours. **Results:** Dot blot analysis detected specific EV markers CD63 and CD81, and nanoparticle tracking analysis (NTA) showed that EV size was <160nm. The EVs promoted the release of TNF-α, IL-6, IL-8 and IL-1β, but not IL-10, in a dose-dependent manner, without affecting the cellular viability, as evaluated by LDH and XTT methods. EVs promoted an increase of NF-kB phosphorylation, whose inhibition reduced the cytokine production, suggesting the participation of this transcription factor in the EV proinflammatory effects. The endotoxin concentration on EV preparations was <0.181 EU/mL (<0.04 ng/mL). **Conclusion:** Our results show that neutrophil-derived EVs act as proinflammatory agents on PBMCs through the NF-kB pathway, suggesting that these EVs are critical mediators of inflammatory responses. Studies are underway to evaluate the expression of cell markers and to determine the cellular subsets producing the inflammatory mediators in response to EV exposure.

320. 197. **IDENTIFICATION OF ANTI-INFLAMMATORY ACTIVITIES OF ACANTHOSPERMUM AUSTRALE THROUGH NETWORK PHARMACOLOGY IN SILICO ANALYSIS AND EXPERIMENTAL VALIDATION**

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Inflammation is essential for homeostasis and defense against injury; however, excessive inflammation can lead to various diseases. Currently used immunomodulatory drugs are useful; nevertheless, they have several disadvantages such as adverse effects or a lack of response to treatment. Ethnopharmacological knowledge of medicinal plants is a valuable resource for the development of novel therapeutic agents. *Acanthospermum australe* has traditionally been used to alleviate inflammation. However, the anti-inflammatory activity of this plant has not been explored. This study aimed to evaluate the immunomodulatory activity of plant extracts by network pharmacology analysis and experimental validation. The compounds in the methanolic extract were obtained from the lotus database. Human molecular targets were predicted using SwissTarget. Using the identified targets, a Venn intersection analysis was performed using a database of human inflammation-related genes. Protein–protein interaction network analysis and cellular pathway enrichment were performed using Cytoscape. Network pharmacology analysis showed that *A. australe* could act at the level of the Toll-like receptor (TLR) pathway and pathway components such as MAPK, PI3K-Akt, and the T-lymphocyte receptor. Considering the identified *in silico* targets/pathways, the expression of inflammatory molecules was assessed using qPCR in THP-1 monocytes stimulated with the TLR4 agonist lipopolysaccharide (LPS). In addition, the effect on T-lymphocyte proliferation in a stimulated murine splenocyte model was evaluated. *In vitro* assays showed that *A. australe* reduced IL-1β, TNF-α, IL-6, and CCL2 expression, and T-lymphocyte proliferation. These results show for the first time the anti-inflammatory effects of *A. australe* and the possible targets of this activity. These results contribute relevant data to validate the traditional use, future preclinical or clinical trials, and the isolation of bioactive molecules.

321. 259. **SEGATELLA COPRI OUTER MEMBRANE VESICLES AS DRIVERS OF BACTERIA-EPITHELIUM-ANTIGEN PRESENTING CELL INTERACTIONS**

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Several studies suggest that *Segatella* (*S.*) *copri*, a member of the gut microbiota, contributes to the immunopathogenesis of rheumatoid arthritis (RA), which is a degenerative autoimmune disease. *S. copri* is overrepresented in preclinical and new onset RA, promotes arthritis in susceptible mice and induces specific lymphocyte responses in RA patients. Nonetheless, the mechanisms involved in the dissemination of *S. copri*-derived antigens to distant tissues are still unknown. Gram-negative bacteria, such as *S. copri,* secrete outer membrane vesicles (OMVs), which are nanosized lipid particles ranging 20-200 nm in size. OMVs of some oral and intestinal bacteria have been shown to transport virulent factors, genetic material and bacterial antigens to peripheral tissues and modulate the function of host (immune) cells. The aim of this study was to elucidate the previously unknown effect of *S. copri*-derived OMVs on the epithelial – immune cell interface. For this purpose, monolayers of intestinal epithelial cell lines HT-29 and Caco-2 were exposed to *S. copri* OMVs and the expression of inflammatory cytokines as well as tight junction genes and proteins was evaluated using RT-qPCR and confocal microscopy. Additionally, we assessed the phenotype and cytokine profile of human monocyte-derived macrophages upon stimulation with *S. copri* OMVs by flow cytometry and ELISA, respectively. Furthermore, endocytosis of DiO-labelled OMVs by epithelial cells and macrophages was determined through confocal microscopy. *S. copri* OMVs were endocytosed by Caco-2 and HT-29 epithelial cells and monocyte-derived human macrophages. Exposure to *S. copri* OMVs reduced tight junction expression in epithelial cells, while stimulating macrophage polarization towards a proinflammatory phenotype and cytokines profile.
Our results suggest that *S. copri*-derived OMVs alter the intestinal epithelial barrier, allowing translocation of *S. copri*-derived factors and macrophage activation, which could promote systemic inflammation driving the development of RA.

322. 263. **THE INTERPLAY OF INFECTIOUS AND METABOLIC-DRIVEN LOW GRADE CHRONIC INFLAMMATION WITH T-CELL IMMUNOSENESCENCE**

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Chronic inflammation is closely related to inflammaging, a low-grade inflammatory state that contributes to immunosenescence (IS). Although the immunosenescence process is part of natural aging, infectious and metabolic-driven chronic inflammatory states can promote an early IS, which could exacerbate the existing conditions. Therefore, our objective was to evaluate parameters related to IS in T-lymphocytes of individuals with chronic Indeterminate Chagas disease (ICh) and/or type-2 Diabetes mellitus (D2). Sex- and age-matched patients (40–60 years old) and healthy volunteers (Co) were recruited from Centenario Hospital in Rosario, Argentina, with 10–15 participants per group. The evaluation from PBMCs included the analysis of T-cell receptor excision circles (SjTRECs) as an indicator of thymic functionality (by qPCR), as well as, senescence, activation and memory profiles of $CD4^+$ and $CD8^+$ populations by flow cytometry. Results indicate a significant reduction of SjTRECs and naïve T-cells in ICh+D2 individuals compared to ICh and Co ($p<0.05$ in both cases). Patients with ICh+D2 showed enhanced effector memory $CD4^+$T-cells frequencies ($CD45RA^-CCR7^-$), and a cytotoxic ($CD4^+CD107a^+$) and activated profile ($CD4^+$HLA-DR^+) ($p<0.05$ vs Co). ICh+D2 also showed an enhancement of $CD8^+$T-cell activation (HLA-DR^+) and TEMRA ($CD8^+CD45RA^+CCR7^-$) frequencies ($p<0.05$ vs Co). All patients tend to increase aging markers ($CD28^-/CD57^+$), compared to Co, being more enhanced in D2 and ICh+D2 individuals [mean±SEM, (%); $CD4^+CD28^-$: Co=7.76±1.0; ICh=9.01±0.8; D2=10.0±2.2; ICh+D2=11.0±2.0/ $CD8^+CD57^+$: Co=40.6±4.4; ICh=43.4±3.5; D2=55.9±4.6; ICh+D2=53.0±1.6]. Our findings showed that comorbidities are associated with more increased IS parameters. Future studies, including larger cohorts of patients, may provide more information on the interaction between the inflammatory response associated with both pathologies and immunosenescence parameters.

323. 277. DECREASED CHOLINERGIC SIGNALING MODULATES LUNG INJURY AND MORTALITY INDUCED BY INTESTINAL ISCHEMIA AND REPERFUSION (I/R) IN MICE.

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Background: Intestinal ischemia and reperfusion (I/R) is a significant risk factor for the development of extrapulmonary acute respiratory distress syndrome, which can induce death under severe conditions, particularly due to inflammation. The cholinergic pathway is crucial in controlling lung inflammation. Objective: To evaluate the role of the cholinergic pathway in a model of I/R-induced acute lung injury in mice. Methods: Male mice with reduced vesicular acetylcholine transporter levels (VAChT-KD^{HOM}) or wild-type controls (WT) were submitted to I/R followed by 2 hours of reperfusion. SHAM animals were not submitted to I/R. Some mice were treated with PNU-282987, an alpha7 nicotinic acetylcholine receptor (α7nAChR) agonist. Mortality rate, lung parenchyma integrity, and inflammatory cell recruitment were assessed. Results: 40% of VAChT-KD^{HOM} mice died during reperfusion, while none of the WT mice did. I/R and VAChT-deficiency reduced the intact parenchyma area and increased the distended or collapsed parenchyma areas ($p<0.001$ and $p<0.01$, respectively). Additionally, I/R caused inflammation and hemorrhage in the lungs of VAChT-KD^{HOM} mice, as evidenced by increased mononuclear cells ($p<0.001$) and red blood cells ($p<0.0001$), respectively. PNU treatment reduced polymorphonuclear cells in the lungs of WT mice ($p<0.05$). Although PNU had no significant effect on mononuclear cell levels and hemorrhage in VAChT-KD^{HOM} mice, it protected against I/R-induced parenchyma damage in both groups, pre-

venting lung distension and collapse (p<0.0001). Most importantly, PNU treatment completely prevented VAChT deficiency-induced mortality. Conclusion: Decreased cholinergic signaling intensifies lung damage and induces mortality in mice submitted to I/R, an effect that was completely prevented by α7nAChR activation.

324. 282. **PLASMA EXTRACELLULAR VESICLES PROMOTE TISSUE REPAIR ASSOCIATED WITH INFLAMMATION RESOLUTION**

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Plasma extracellular vesicles promote tissue repair associated with inflammation resolution. Plasma extracellular vesicles (pEVs) have pathological and physiological effects, but their physiological role on macrophages, fibroblasts and endothelium are poorly reported. We demonstrated that pEVs play a relevant role controlling inflammation, by reducing IL-6 and TNF release in PAMP-estimulated macrophages and increasing IL-10 secretion. We hypothesize that pEVs have pro-resolving effects in macrophages, endothelium and fibroblasts. 2ml of PGI treated plasma were used to purify pEVs by SEC and centrifuged at 30k xg at 4°C for 90 min. Identity and purity of EVs was confirmed by immunoblot against CD63, CD81, CD9, ALIX and HSP70 and contaminants like APOA1, APOB100 and IgG. By NTA we determined a size of 200nm and a concentration of 4X10^7 pEV/ml. Addition of pEVs + Resiquimod (R) to macrophages increased the expression of pro-resolving markers: VEGFa (p<0.001), CD300e (p<0.001), RGS2 (p<0.00001), CD93 (p<0.05) and TIMP-1, promoted the expression of HB-EGF (p<0.05), SERPIN-E1 (p<0.001) and decreased SERPIN-F1 (p<0.001). Supernatants of macrophages stimulated with pEVs triggered tubulogenesis (p<0.001) and reduced MMP9 activity (p<0.001). pEVs promoted wound closure in a wound healing (WH) assay of endothelial cells and fibroblasts. Regarding ELISAs, when HUVECs were stimulated with LPS+pEVs, they significantly increased their release of il8 (p<0.001) and il6 (p<0.05). Additionally, macrophages supernatant, both in the costimulation with LPS or R, pEVs increased VEGF release (p<0.001). Finally, macrophages stimulated with R+pEV enhanced their efferocytic activity (p<0.05). Our assays were quantified using ANOVA or T-test. We conclude that pEVs from healthy donors promote a resolutive phenotype by acting on important cell types for tissue repair. By continuing our investigation of pEVs on these cells and then, on *in vivo* models, we hope to improve our understanding of their functions and, potentially, laying the foundations for future regenerative therapies based on pEVs.

325. 304. **CHARACTERIZATION OF MUSCLE DAMAGE AND EXUDATE PRODUCED BY DIFFERENT METALLOPROTEINASES FROM BOTHROPS ATROX SNAKE VENOM**

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Background: In Brazil, 25,000 snakebites occur per year and *Bothrops atrox* is responsible for the majority of accidents. The morbidity from local effects is high and they are mainly resulted by the action of snake venom metalloproteinases (SVMPs). The major SVMPs in *B. atrox* venom are Atroxlysin-Ia and Bathroxragin. Both are highly efficient in hydrolyzing ECM proteins, inducing rapid haemorrhage/dermonecrosis. Peptides generated after *in vitro* hydrolysis of ECM induces inflammation in mice. **Objectives:** Characterization of the exudate produced after SVMPs injection into the mice gastrocnemius muscle and its ability to activate/stimulate inflammatory responses in cells cultures. **Methods:** Muscle damage was evaluated by CK levels and histological analysis. The composition of the exudate was analysed by mass spectrometry and inflammatory mediators were quantified using CBA kit. Mediators from cell culture supernatant (C2C12/J774) treated with exudate were measured by CBA and gene expression by real-time PCR. **Results:** The SVMPs induced disorganization of muscle fibers and migration of inflammatory cells, in ad-

dition to increasing CK levels in the plasma. The proteomic characterization showed presence of collagen, fibrinogen, plasminogen, vitronectin and fibronectin. The main DAMPs/immunomodulators identified on exudate fluids were proteins from heat shock family (HSP), protein S-100 and annexin. The exudate presented moderate levels of IL-10, TNF-alpha, IL-2, IL-4 and a high concentration of IL-6. The exudate from both toxins induced production of inflammatory mediators by macrophages and muscle cells (myoblasts and myotubules). The cytokines with the highest levels detected were TNF-alpha, mainly in J774 and myoblasts. IL-6 was very high for the three cultures evaluated. The increase in gene expression of pro-inflammatory molecules also was observed for both exudates and again IL-6 was very high for all cells. **Conclusion:** The hydrolysis products of SVMPs triggers an exacerbated inflammatory response and this may be one of the reasons why anti-venom is not able to neutralize local effects.

326. 339. **EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AND PROSTAGLANDIN E2 (PGE2): A LINK BETWEEN INFLAMMATION AND CERVICAL CANCER**

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Background: Cervical cancer (CC) is the fourth most common tumor in women worldwide. Cisplatin-based chemotherapy is broadly used, yet survival rates remain poor, needing new therapeutic strategies. The epidermal growth factor receptor (EGFR) is highly expressed in solid tumors, being associated with different cellular functions. Previous research showed that EGFR induces cyclooxygenase-2 (COX-2) expression in CC, resulting in prostaglandin E2 (PGE2) production. Studies suggest that PGE2 transactivates EGFR in some models. **Objectives:** This work investigated the crosstalk between EGFR and PGE2 signaling pathways in CC and its pro-tumor effects. **Methods:** EGFR, COX-1, COX-2, and mPGES-1's roles in CC gene expression were evaluated using The Cancer Genome Atlas (TCGA). *In vitro* experiments used aggressive (HeLa and CASKI) and non-aggressive (C33A) cell lines. Panitumumab inhibited EGFR, and aspirin inhibited COX-1/-2. Cell migration was evaluated using the Boyden chamber. MTT and clonogenic assays determined cell viability and clonogenicity, respectively. Western Blot analyzed protein expression/phosphorylation. PGE2 was measured in the lineages using an Elisa kit for Human PGE2. **Results:** TCGA analysis revealed a positive correlation between EGFR and PGE2 biosynthesis pathway genes. CC patients showed decreased overall survival upon overexpression of EGFR or COX-2. CASKI and HeLa cells were cisplatin-resistant, while C33A was sensitive. CASKI cells expressed the highest basal levels of EGFR, while HeLa showed elevated COX-2 and mPGES-1 expression. HeLa cell line showed higher COX-2 expression during the EGF activation kinetics, which was reversed with EGFR inhibition, in protein expression and in the supernatant dosage. PGE2 activated the ERK/MAPK signaling, increasing CC cell motility. Association of panitumumab with aspirin decreased CC cells viability, colony formation and also sensitized CASKI cells to cisplatin chemotherapy. **Conclusions**: EGFR-PGE2 pathway interaction links inflammation and oncogenic signaling, promoting aggressive CC behavior.

327. 410. **INFLAMMAIDS: EXPLORING THE INTERPLAY BETWEEN METABOLIC SYNDROME AND CHRONIC INFLAMMATION IN CHILEANS LIVING WITH HIV**

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Background: Chile faces significant public health challenges, with high rates of metabolic syndrome (MetS) and over 91,000 individuals living with HIV

(PLWH), as estimated by UNAIDS. Approximately 50% of PLWH also suffer from MetS, which exacerbates chronic inflammation and complicates health outcomes. **Aim:** To characterize the interplay between MetS and chronic inflammation in Chilean PLWH. **Methods:** The InflammAIDS prospective cohort study enrolled PLWH on stable antiretroviral therapy with undetectable viral loads (for at least six months) and HIV-negative participants (HN). Data were collected on demographics, medical history, and laboratory results. Participants were categorized as MetS- or MetS+ according to WHO criteria. Ethical approval was obtained from the Scientific Ethical Committee of the Chilean Ministry of Health. Statistical analyses included two-way ANOVA and Student's t-tests, as appropriate. **Results:** 106 PLWH and 34 HN were enrolled, predominantly male (97.14%), with a mean age of 44.3± 10.1 years and mean CD4+ counts of 647±272 cells/mm^3. MetS+ groups (PLWH and HN) showed higher waist circumference, triglyceride, and HbA1c levels than their respective MetS- groups. Additionally, increased scores on several metabolic indices were observed, suggesting similar cardiovascular risk profiles. The PLWH MetS+ group also displayed higher hs-CRP serum levels than the PLWH MetS- group, which indicates heightened systemic inflammation. Leukogram analysis revealed higher monocyte and lower neutrophil percentages in the PLWH MetS+ group compared to HN MetS+, with reduced neutrophil percentages in both PLWH groups versus their HN counterparts. Flow cytometry analysis of blood samples from PLWH groups did not reveal significant differences regarding counts or percentages of CD3+, CD4+, or CD8+ lymphocytes, nor respecting CD4/CD8 ratios, a recognized positive prognostic marker in PLWH. **Conclusion:** These findings suggest that Chileans living with HIV and MetS display distinct alterations in neutrophil and monocyte frequencies compared to Chileans living with MetS without HIV.

328. 439. **FISH OIL SUPPLEMENTATION IMPROVED GLYCEMIC PROFILE WITH FABP4 GENE EXPRESSION REDUCTION IN BONE MARROW CELLS IN SPONTANEOUS TYPE 2 DIABETES GOTO-KAKIZAKI RATS**

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Type 2 diabetes (T2D) has negative effects on skeletal health. Hyperglycemia and insulin resistance are associated with impaired bone marrow (BM) homeostasis including expansion of adiposity and decreased bone formation, one of the key pathogenic mechanisms underlying osteoporosis and bone fragility in T2D. Fish oil (FO) supplementation may play a critical role in the homeostasis of BM cells by altering the energy metabolism and the differentiation fate of mesenchymal stem cells (MSC) into adipocyte or osteocytes. We hypothesize that T2D increases BM adiposity independent of obesity which may be modulated by FO supplementation. Eight-weeks-old male Wistar rats (WT) and male Goto-Kakizaki rats (GK), a spontaneous model of T2D in lean animal, were divided into four groups, two of which received water (WTw and GKw) and the other two received FO (WTn-3 and GKn-3). Water or FO supplementation (5.4:1 EPA/DHA, HiOmega 3, Naturalis) was administered by oral gastric gavage, 2 g per kg body mass, three times a week for eight weeks. We performed fasting blood glucose, glucose and insulin tolerance test. After harvesting the BM from femurs and tibias, we isolated mononuclear cells using density gradient and then isolated mRNA for polymerase chain reaction (qPCR,) analysis. The expressions of genes that participate on MSC differentiation in adipocytes (Wnt5b, PPAR-gamma, FABP4) were investigated using the Rplp0 gene expression as endogenous. GK rats showed fasting hyperglycemia, glucose intolerance and insulin resistance. All these three glucose parameters were attenuated in GK rats supplemented with FO. GK rats demonstrated increased expression of PPAR-gamma, FABP4 and Wnt5b, genes related to adipogenesis. FO supplementation reduced the gene expression of FABP4 in BM mononuclear cells of GK rats. In conclusion, fish oil has the potential for glycemic control and attenuation of BM adipogenesis, having the beneficial effect to be a non-drug intervention for patients with T2D.

329. 499. **SIRT6 EXPRESSION IS RELATED TO PROINFLAMMATORY CYTOKINES AND 5-FLUOROURACIL SENSITIVITY IN COL-**

ORECTAL CANCER MICE MODEL

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Colorectal cancer (CRC) is the most common cancer in the digestive tract, accounting for about 10% of cases worldwide. Its development is influenced by various intrinsic and external factors, including chronic inflammation conditions like ulcerative colitis and Crohn's disease. Epigenetic regulation plays a crucial role in CRC by promoting or controlling carcinogenic processes. Key mechanisms include DNA methylation, histone modification, and the activity of non-coding RNAs. These epigenetic marks are studied as potential biomarkers for early CRC detection, diagnosis, and prognosis. Sirtuins (SIRT), a family of NAD-dependent histone acetylase proteins, regulate several biological processes, such as inflammation, metabolism, oxidative stress, and apoptosis. Among them, Sirtuin 6 (SIRT6) is particularly interesting in CRC. In previous reports, high levels of SIRT6 have been linked to poor prognosis and low survival rates in CRC, though its precise role remains unclear. Nonetheless, SIRT6 may promote inflammation by increasing cytokine production, contributing to cancer progression and angiogenesis. Chemoresistance in CRC is a significant challenge, and research using a mouse model of colitis-associated colorectal cancer (CAC) has shed light on the signaling pathways involved. Experimental therapies, including those using molecules secreted by *Taenia crassiceps* (TcES), have demonstrated anti-tumor effects on CAC. This study aimed to evaluate SIRT6 and proinflammatory cytokines mRNA and protein expression from mouse CAC treated with 5-FU monotherapy, TcES, and combined therapy. The results showed increased SIRT6 expression in samples from untreated CAC mice, while treatments, particularly those involving TcES, reduced SIRT6 levels. Additionally, changes in the expression of proinflammatory cytokines like TNF-alpha, IL-8, and IL-1-beta were observed. These findings suggest that SIRT6 expression is related to regulating proinflammatory cytokines, which plays a significant role in CAC development and resistance to chemotherapy, while TcES treatment negatively regulates SIRT6 expression and improves the therapy response.

330. 534. **EVALUATION OF CARDIOVASCULAR RISK BIOMARKERS IN ACUTE POST-COVID-19 SYNDROME**

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Background: COVID-19 is an illness caused by the SARS-CoV-2 virus that has affected millions of individuals worldwide. The World Health Organization (WHO) estimates that 10-20% of infected individuals may develop chronic disease, with the likelihood rising to 50-70% among those with moderate to severe cases. This chronic condition, known as acute post-COVID-19 syndrome (PACS), is characterized by the persistence of symptoms and presents significant diagnostic challenges due to its multisystemic nature. Among the affected systems, the cardiovascular system is notably impacted, with the development of cardiovascular diseases being a potential sequel of PACS. However, the cardiovascular pathophysiological mechanisms are not well understood in this syndrome and the parameters related to cardiovascular risk need to be investigated. **Objectives:** The present study aims to evaluate cardiovascular risk biomarkers associated with lipid and thromboinflammatory profiles and to investigate their correlation with the development of cardiovascular diseases in PACS. **Methods:** Following approval by the research ethics committee, the study included 31 survivors of moderate to severe acute COVID-19 with symptoms lasting from 30 days to 6 months, and 19 control volunteers with negative RT-PCR results for SARS-CoV-2. Lipid profiles were assessed using quantitative colorimetric assays, while thromboinflammatory profiles were analyzed using the ELISA technique. **Results:** The study revealed that survivors exhibited persistent symptoms, particularly those affecting the cardiovascular system. Laboratory results indicated elevated levels of non-HDL-c and LDL-c, alongside increased concentrations of IL-6, IL-1beta, CRP, fibrinogen, and D-dimer, with a concomitant reduction in HDL-c levels. Furthermore, there was an observed increase in cardiovascular indices associated with lipid and thrombotic profiles. **Conclusion:** Our findings suggest persistent dyslipidemia, systemic inflammation, and hypercoagulability, which may contribute to heightened cardiovascular risk and the pathophysiology of cardiovascular diseases in PACS.

331. 587. **CHARACTARIZATION OF THROMBOINFLAMMATION DURING CHIKUNGUNYA INFECTION**

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Introduction: Chikungunya fever, caused by the Chikungunya virus (CHIKV) and transmitted by Aedes mosquitoes, presents with acute symptoms like fever, myalgia, rash, and severe arthralgia. In 30-50% of cases, joint pain can persist and become severe, potentially leading to significant functional disability. Growing evidence suggests that immune activation and inflammatory mediators contribute to the progression of severe disease. Recently, platelets have been recognized as central players not only in hemostasis but also in the response to viral infections, linking inflammatory and thrombotic responses—an event known as thromboinflammation, which is significant in some arboviral diseases. **Objective:** This study aims to characterize the role of platelets in thromboinflammation and their influence on the chronicity of Chikungunya fever. **Methods and Results:** We analyzed plasma from 132 Chikungunya patients and 25 healthy volunteers in Rio de Janeiro, Brazil. Our findings indicate that Chikungunya infection leads to significant platelet activation, as shown by increased CD62P expression and elevated platelet-derived inflammatory mediators. Additionally, platelet activation and soluble p-selectin at symptom onset were associated with the development of chronic disease. Infected patients' platelets also showed elevated levels of NLRP3, caspase 4, and cleaved IL-1β, suggesting inflammasome involvement. In vitro experiments confirmed that CHIKV directly activates platelets. Proteomic analysis revealed changes in proteins related to platelet activation, cell death, and antiviral response. ELISA plasma analysis showed distinct cytokine release patterns, including CXCL10/IP-10, CCL2/MCP-1, and IL-1ra, alongside elevated levels of FVW, CD-62P, TFPI, and PAI-1. High VCAM-1 and ICAM-1 expression indicated endothelial activation. CHIKV-activated platelets also showed increased platelet-monocyte aggregates and modulated the monocyte inflammatory response. In conclusion, platelets are activated during Chikungunya infection and may contribute to the immune and inflammatory processes. Further studies are needed to understand their role in the long-term effects of the infection.

332. 608. **UNFOLDED PROTEIN RESPONSE FACTOR ATF6 AND XBP1 INCREASE THE INFLAMMATORY CYTOKINE EXPRESSION IN DENDRITIC CELLS AND AUGMENTS THE SYSTEMIC INFLAMMATORY RESPONSE INDUCED BY TLR AGONISTS IN HIGH SATURATED FATTY ACID CONTEXT**

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Background. Dendritic cells (DCs) are key in the coordination of adaptive immunity mediated by T lymphocytes (LT). The LT-priming require specific cytokines derived from activated-DCs, and this activation is partially support by the unfolded protein response (UPR), a cellular mechanism that regulates the fidelity of the cellular-proteome. The UPR axis is regulated by ATF6, XBP1 and PERK, which can induce the expression of inflammatory factors in infectious settings or in high fatty acid environments, but their overall contribution over DCs functions and metabolism is largely unknown. Additionally, the increase of physiological palmitic acid (PA) promotes a hyper-inflammatory status in response to LPS, and DCs exposed to PA plus TLR agonists show an impairing glycolysis and cytokine overexpression. In this research, we evaluated the contribution of ATF6 and/or XBP1 over DCs glycolysis and cytokine expression in the hyper-inflammatory status promoted by high fatty acid plus TLR agonists in transgenic mice ATF6$^{\Delta DC}$, XBP1$^{\Delta DC}$ and ATF6/XBP1$^{\Delta DC}$ conditional knock-out in DCs.
Method. The ATF6 and/or XBP1 physiological contribution was performed in an acute inflam-

mation setting mediated by high fatty acid (PA) and LPS in ATF6$^{\Delta DC}$, XBP1$^{\Delta DC}$ and ATF6/XBP1$^{\Delta DC}$ transgenic mice (C57BL/6 background). GMCSF-derived DCs was established from the same transgenic mice, which has been activated with TLR7-agonist plus PA. The activity of the UPR, cytokines and glucose metabolism in DCs were analyzed by qPCR and flow cytometry assay (SCENITH, CBA). **Result and conclusion.** The serum cytokines IL-6, TNFα, MCP1/CCL2 decreased significantly in acute systemic inflammation setting mediated by high fatty acid and LPS in ATF6$^{\Delta DC}$ and XBP1$^{\Delta DC}$, but not in ATF6/XBP1$^{\Delta DC}$ mice. The deficiency of ATF6 or XBP1 decrease the IL-6 and IL-12 expression, while IFNγ, IFNβ and IL-10, were regulated differentially in DCs. Curiously, inflammatory cytokines were not reduced in ATF6/XBP1$^{\Delta DC}$ and the PERK axis was overregulated. Finally, the deficiency of ATF6 or XBP1 increase the glycolytic capacity in DCs. In conclusion, the UPR is an emerging actor in inflammation mediated by DCs.

333. 628. EXPLORING THE COMPLEMENT SYSTEM'S ROLE IN ACUTE KIDNEY INJURY TRIGGERED BY BOTHROPS JARARACA VENOM

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The clinical manifestations of snake envenomation are complex, encompassing local effects such as pain, edema, ecchymosis, blisters, abscesses, and necrosis. Systemic effects include hemorrhage, coagulopathy, shock, hypotension, oliguria, and acute kidney injury (AKI), which is the most severe complication. AKI is characterized by anuria and elevated serum creatinine levels, potentially necessitating dialysis and representing the leading cause of death among survivors of initial envenomation effects. While antivenom is the only scientifically validated treatment, it neutralizes venom toxins but does not target the inflammatory mediators in damaged tissues. Recent studies indicate that *Bothrops jararaca* venom may activate the complement system, potentially exacerbating symptoms of envenomation. This project aims to analyze the interaction between *B. jararaca* venom and the complement system on human renal cells. An *ex vivo* human whole blood model was utilized alongside renal cells derived from the proximal tubule (HK-2 cell line). Results demonstrate that *B. jararaca* venom induces complement system activation in the human whole blood model, significantly increasing the production of anaphylatoxins, including C3a, C4a, C5a, and sTCC. Venom exposure also leads to the production of inflammatory cytokines and chemokines, such as TNF-α, CXCL8, CCL2, CXCL9, and CCL5. Furthermore, *in vitro* assays showed that HK-2 cells treated with human plasma from the whole blood model, as source of complement molecules, exhibited elevated levels of inflammatory cytokines and chemokines, including IL-6, CXCL8, and CCL5. These findings underscore the role of the complement system in mediating the inflammatory response associated with *B. jararaca* venom exposure and suggest potential therapeutic strategies that target both venom neutralization and modulation of complement activation to mitigate renal damage.

334. 630. NOVEL BACTERIAL CLUSTER PREVOTELLA, BACTEROIDES AND SUTERELLA IS ASSOCIATED WITH MORTALITY IN MEXICAN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE (ACLF) AND THE CLINICAL UTILITY OF SYSTEMIC HS-CRP AND IL-6: AN INNOVATIVE APPROACH INVOLVING MASSIVE NEXT-GENERATION SEQUENCING AT INTESTINAL LEVEL

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Background: ACLF is a syndrome characterized by acute decompensation of cirrhosis, organ failure, and high short-term mortality. Currently, the study of intestinal microbiota (IM) is relevant

to understand the pathophysiology of cirrhosis. However, there are no studies focused on IM in alcohol-associated ACLF in Mexican population and its relationship with mortality and inflammatory markers; therefore, these were the objectives of the study. **Material and methods**: Cross-sectional analytical study, which included 22 patients with ACLF, 16 without ACLF (DC), and 18 healthy controls (HC), recruited at the Hospital Civil de Guadalajara. IM was characterized in fecal samples by NGS sequencing of the 16S-rRNA gene. Serum levels of hs-CRP and IL-6 were quantified by ELISA and bioinformatics analysis of IM was performed using QIIME2. **Results**: The bacterial profile in ACLF was dominated by pathogenic/inflammatory genus such as *Escherichia*/*Shigella*, *Enterobacter* and *Prevotella*. Interestingly, subanalysis of IM in ACLF categorized at
7 and 90 days of mortality showed enrichment of *Prevotella*, *Bacteroides* and *Suterella* clusters. Serum levels of hs-CRP and IL-6 were increased in ACLF. hs- CRP levels positively correlated with IL-6, whereas *Firmicutes*/*Proteobacteria* ratio negatively correlated with α-diversity. Hs-CRP allows discrimination of infections in CD patients using a cut-off point >70.7 mg/L (90% sensitivity and 68.9% specificity). IL-6 allows discrimination of hepatic encephalopathy (HE) in patients with CD and ACLF with a cut-off point >7051.1 pg/mL (81.4% sensitivity and 45.8% specificity). **Conclusions**: The dysbiotic/proinflammatory profile of IM in ACLF correlated with increased systemic inflammation. The bacterial cluster *Prevotella*, *Bacteroides* and *Suterella* is a marker of mortality at 7 and 90 days. IL-6 and hs-CRP allow discrimination of HE and infections in patients with alcohol-associated cirrhosis.

335. 631. **EFFICACY OF NUTRACEUTICALS IN ENHANCING PERIODONTAL THERAPY: A SYSTEMATIC REVIEW OF IMMUNE AND CLINICAL MODULATION**

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Background and aim: Currently, has been growing the interest in effects of immunologically active molecules derived from food or microorganisms, often termed nutraceuticals in the periodontal disease. The aim of this study is to assess the efficacy of nutraceuticals as an adjunct of mechanical periodontal therapy (MPT), comparing their effects to placebo on clinical and immunological outcomes. Methods: A comprehensive literature search was conducted in PubMed and Embase using MeSH, DeCS, and Emtree terms in March 2024. Inclusion criteria included adults with gingivitis or periodontitis, double-blinded randomized controlled trials , and evaluations of nutraceuticals versus placebo in periodontal therapy. Of the 62 publications screened, 13 met the eligibility criteria. Results: Selected studies of nutraceutics showed that vitamin D supplementation, combined with MPT, significantly reduced CD3+CD8+ cytotoxic T lymphocytes and enhanced autophagy-related protein expression in mononuclear cells. Resveratrol modulated inflammatory responses in diabetic patients with periodontitis. The reduction in pro-inflammatory cytokines like IL-1α and MCP-1 in the test group but it did show improved serum IL-6 levels. Bilberry consumption significantly reduced gingival inflammation,decreased bleeding on probing and lower levels of pro-inflammatory cytokines, including IL-1β, IL-6, and VEGF, in the gingival crevicular fluid. Supplementary probiotic consumption affected the oral microbiome and immune response, as evidenced in the relative abundance of bacterial genera and salivary cytokine levels. Bifidobacterium lactis HN019 reduced plaque accumulation and gingival bleeding while influencing the expression like beta-defensin-3, toll-like receptor 4, CD-57, and CD-4. Symbiotic supplements with MPT, decreased IL-1β, MDA, plaque index, pocket depth, and clinical attachment loss, while increasing antioxidant capacity. Symbiotic tablets effectively reduced clinical and biochemical inflammation markers. These findings underscore the anti-inflammatory potential of both bilberries and synbiotics. Conclusions: Nutraceutical treatments can significantly impact the immune system by reducing inflammation, modulating immune T cell activity, and enhancing the body's ability to combat periodontal pathogens.

336. 636. **MESENCHYMAL STEM CELL EXTRACELLULAR VESICLES ATTENUATE DENDRITIC CELL MATURATION IN VITRO**

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Background: In inflammatory states, dendritic cells infiltrate bone tissue and activate T cells, increase osteoclast differentiation, and subsequently increase bone resorption and destruction. Therefore, the control of dendritic cell maturation is imperative to potentiate repair. The concept of mediating tissue regeneration using transplanted mesenchymal stem cells (MSCs) was primarily based on the early hypothesis that the cells would engraft and differentiate, to replace damaged tissue. However, low engraftment rates and poor survival remain a barrier for exploitation of the multipotency of MSCs. Despite this, tissue regeneration is still potentiated. These observations gave rise to the developed hypothesis that MSCs facilitate tissue regeneration via a paracrine method, through secretion of soluble factors and extracellular vesicles (EVs). EVs are secreted membrane bound nano-vesicles that contain proteins, lipids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). EVs are found in most biological tissues and are involved in cargo transfer between cells.
Objective: It is hypothesized here that MSC-EVs are phagocytosed by dendritic cells, and this subsequently attenuates dendritic cell maturation. The aim of this study was to evaluate the effect of MSC-EVs on dendritic cell maturation *in vitro.* ***Methods:*** EVs were isolated from the conditioned media of the D1 ORL UVA MSC cell line by differential centrifugation. Immature dendritic cells were differentiated from C57BL/6 bone marrow monocytes in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF). Dendritic cell maturation was induced via lipopolysaccharide (LPS) and CpG oligonucleotide stimulation. Mature dendritic cells were subsequently treated with MSC-EVs and maturation and migration were assessed via flow cytometry. ***Results:*** MSC-EVs are phagocytosed by dendritic cells, and this is potentiated by LPS CpG oligonucleotide stimulation. MSC-EVs reduce the surface marker expression of CD80, CD83, CD86, CD40 and MHC II and the migratory properties of LPS CpG oligonucleotide-stimulated dendritic cells. ***Conclusion:*** MSC-EVs attenuate maturation in LPS CpG oligonucleotide-stimulated dendritic cells.

337. 686. EFFECTS OF CRYOTHERAPY ON INTERLEUKINS-6 IN TRAINED INDIVIDUALS: A SYSTEMATIC REVIEW OF RANDOMIZED CONTROLLED TRIALS WITH META-ANALYSIS AND GRADE ASSESSMENT

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Background: Athletes often use cryotherapy modalities to optimize recovery and enhance performance by potentially reducing inflammatory markers like interleukin-6 (IL-6). IL-6 is an inflammatory biomarker following intense exercise, and its reduction is believed to represent recovery in the short term. Despite its popularity, there is limited systematic review and meta-analysis on cryotherapy's impact on IL-6 levels after physical training. ***Objectives:*** This systematic review aimed to evaluate cryotherapy's immediate and short-term effects on IL-6 levels in trained individuals. ***Methods:*** Searches were performed across PubMed, Embase, Central, Physiotherapy Evidence Database (PEDro), PsycINFO, SportDiscuss, and Cinahl without restrictions on publication date or language. Trials were independently selected, data extracted, and risk of bias assessed by two reviewers, with discrepancies resolved by a third. The 0-10 PEDro Scale was used for risk of bias evaluation, and meta-analysis was conducted using random-effects models, presenting results as mean differences (MDs) with 95% confidence intervals (CIs). The quality of evidence was assessed using the GRADE approach. ***Results:*** The search retrieved 3,694 references (341 duplicates), of which 27 were selected for full-text, and nine randomized controlled trials (RCTs) were included. The risk of bias ranged from 3 to 6 points (median = 4). Meta-analysis of six studies (n = 121) showed a significant immediate effect of cryotherapy on IL-6 (MD = -0.42, 95% CI -0.85 to -0.00; p = .05). However, no significant difference was found in the short term (MD = -0.33, 95% CI -0.68 to 0.03; p = .07; n = 138). The GRADE assessment indicated Very Low certainty of evidence. ***Conclusion:*** The evidence on cryotherapy's effectiveness in reducing IL-6 levels immediately after exercise in trained individuals is Very Low quality. While there may be an immediate effect, results should be interpreted cautiously, considering the evidence's overall quality and other

inflammation biomarkers.

338. 700. **HEAT-INSOLUBLE CRYOGLOBULINEMIA IN A PATIENT WITH POST-VACCINATION VASCULITIS**

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Background: Cryoglobulins are immunoglobulins or immune complexes that precipitate at temperatures below 37°C. This reversible condition is confirmed through laboratory testing. Persistent cryoglobulinemia is associated with a wide range of clinical manifestations, often affecting the skin, kidneys, and peripheral nervous system. Most cases are secondary to underlying conditions such as monoclonal gammopathy, chronic lymphocytic leukemia, chronic viral infections, or systemic autoimmune diseases. ***Objectives***: To describe a case of a patient with leukocytoclastic vasculitis following influenza vaccination who presented with heat-insoluble cryoglobulinemia. ***Methods***: The recommended protocol for cryoglobulin processing was followed, but no redissolution was observed at 37°C. The serum was subsequently heated to 56°C, a procedure previously reported for this phenomenon to verify redissolution. Depolymerization treatment with Dithiothreitol was performed, followed by immunofixation typing on an Interlab G26 automated electrophoresis system. ***Results***: A 67-year-old woman with a history of untreated chronic lymphocytic B-cell leukemia developed cryoglobulinemic vasculitis as an adverse event following influenza vaccination. Relevant laboratory findings included: C3: 50 mg% (70-165), C4: 2 mg% (14-37), RF: 786 UI/ml (0-20), an oligoclonal pattern in serum protein electrophoresis and mixed type II cryoglobulins according to the Brouet classification. Cryoglobulins were heat-insoluble (not soluble at 56°C) with a cryocrit of 4%. Based on the laboratory report, the patient was diagnosed with cryoglobulinemic vasculitis due to mixed type II cryoglobulins. ***Conclusion***: The finding of heat-insoluble cryoglobulins is an atypical condition with biochemical interest, as it is rarely described in the literature and requires unusual laboratory procedures. Additionally, the failure to redissolve at 37°C can lead to false-negative results, impacting patient diagnosis, management, and treatment.

339. 721. **CHONDROPROTECTIVE AND REGENERATIVE EFFECT OF EXTRACELLULAR PRODUCTS FROM GLYCOLYTIC MESENCHYMAL STEM CELLS**

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6. Centro de Investigación e Innovación Biomédica, Universidad de Los Andes, Santia-go, Chile. IMPACT

Background: Osteoarthritis (OA) is a degenerative disease affecting articular cartilage with no cure. Mesenchymal stem/stromal cells (MSC)-derived small extracellular products (EPs) appear as the candidate of choice for OA treatment due to their chondroprotective properties. However, new strategies are needed to improve their therapeutic efficacy, since they have not reached optimal clinical outcomes. Methods: We propose the use of extracellular products (Eps) derived from metabolically reprogramed glycolytic-MSC (MSC-Glyco) to improve their therapeutic properties. For that purpose, EPs were isolated by ultracentrifugation and then added to chondrocytes from OA patients. The expression levels of hyaline and fibrotic-OA chondrocyte markers were measured by RT-qPCR. Moreover, a collagenase mouse model of OA (CIOA) was used to assess the clinical effect of the intra-articular injection of EPs-MSC-Glyco through MicroCT and histopathological analysis. Results: We show that EPs-MSC-Glyco increase the level of hyaline chondrocyte markers (Collagen 2 and Aggrecan) while reducing fibrotic-OA chondrocytes markers (ADAMTS4 and Collagen 1). Moreover, CIOA mice treated with EPs-MSC-Glyco showed a significant reduction in bone mineral density and in histological joint damage, compared to EPs-MSC and CIOA mice. Conclusion:These results show that EPs-MSC-Glyco represents a potential new strategy for treating OA.

INNATE IMMUNITY

340. 050. OPTIMIZATION OF IN VITRO EXPANSION OF PERIPHERAL BLOOD HUMAN NK CELL

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Background: NK cells are the most important cell population in the anti-tumoral and anti-viral innate response. The study of these cells thru *in vitro* conditions has become crucial. However, the limited number of isolated cells represents a problem. Manufacturers have developed NK-specific growth media combined with the use of IL-2 as the standard for NK cell expansion. Nonetheless, the use of IL-2 requires a high degree of purity in the isolates; otherwise, other populations, such as T lymphocytes could be stimulated and expand in the culture. **Objective:** Analyze the efficacy of a single dose of IL-15 as a complementary cytokine that can improve the quality and purity of the NK cell expansion from peripheral blood. **Methods:** Peripheral mononuclear blood cells were obtained using ficoll-hypaque gradient. NK cells were isolated from mononuclear cells by negative selection using the NK cell isolation kit and expanded with CTS NK Xpander medium supplemented with IL-2 or IL-2/IL-15 and autologous serum. At day 14 of expansion, the number and viability of NK cells were analyzed by flow cytometry using the BD Simultest CD3/CD16+CD56 and BD Pharmigen 7-AAD tests, respectively. Data normality was assessed, followed by ANOVA with Tukey's post hoc, using GraphPad Prism 6. **Results:** NK cells isolated were expanded *in vitro* between 6-7 times using IL-2 or IL-2/IL-15 enriched NK medium. Cell viability was found to be >91% under all conditions. The percentage of NK was higher in the IL-2/IL-15 condition compared to IL-2 (>10%, *P<0.01*); however, no differences were found between the percentage of NK CD56 dim/CD56 bright in both conditions (*P>0.05*). The NK CD56 bright cells population were the most predominant in both conditions. **Conclusion:** In conclusion, the use of IL-15 when combined with IL-2 enriches the expansion of NK cells and prevents T and B cell growth compared with IL-2 alone. 080 Tenacibaculum dicentrarchi modulates the expression of innate immune response genes in Atlantic salmon (Salmo salar) Marcio Aversa Marnai[1], Daniela Espinoza[2], Almendra Benavides[2], Ruth Montero[3], Valeria Silva Álvarez[1], Ana María Ferreira[1], Mónica Imarai[2] 1. Área Inmunología, Facultad de Química, Universidad de la República (Uruguay) 2. Laboratorio de Inmunología, Centro de Biotecnología Acuícola, Facultad de Química y Biología, Universidad de Santiago de Chile (Chile) 3. Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences (Norway) Salmonid aquaculture significantly impacts the global economy by providing one of the major sources of seafood worldwide. Despite the enormous export revenues generated in this activity, it also involves major complications related to environmental management and disease control. The bacteria *Tenacibaculum dicentrarchi* was recently discovered in salmonid farms in Norway and Chile, the leading countries in salmon production globally. This pathogen poses a significant economic threat to salmonid aquaculture and remains a challenging pathogen in the industry, requiring ongoing research and development of effective prevention and treatment strategies to mitigate its impact on fish health and profitability. The present study aimed to elucidate the innate immune mechanisms in Atlantic salmons challenged by *T. dicentrarchi*. With this purpose, a set of 8 immune-related genes was selected to analyze putative changes in their expression upon bacterial challenge in liver, spleen and head kidney, the three tissues with the most immunological relevance. Gene expression of serum amyloid-A protein (*saa*), serotransferrin, lysozyme-C and lysozyme-G, hemopexin-A and hemopexin-B (*hpxB*), haptoglobin (*hp*) and C-reactive/serum amyloid-P protein (*crp/sap*) was studied in salmons at 2 (2 dpc) or 6 (6 dpc) days after *T. dicentrarchi* immersion challenge. The head kidney displayed a moderate modulation of the immune response at 2 dpc, with *crp/sap* and *hpxB* being the most regulated genes (each showing a 15-fold increase). Nevertheless, the most robust changes were observed in the spleen and even more so in the liver at 2 dpc. Interestingly, *hp* and *saa* were the most up-regulated genes in spleen (each showing a 25-fold increase) and also in the liver (8-fold and 100-fold increase, respectively). Our findings provide novel insights into potential early response strategies of salmonids against *T. dicentrarchi*, such as the acute phase response and the regulation of systemic iron metabolism, which is a critical nutrient supply for this patho-

gen.

341. 152. **MODULATION OF NEUTROPHIL EXTRACELLULAR TRAPS RELEASE BY PROVIDENCIA STUARTII AND PROVIDENCIA RETTGERI IN HUMAN NEUTROPHILS**

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Antimicrobial resistance is a major problem for the treatment of infectious diseases worldwide. *Providencia stuartii* (Ps) and *Providencia rettgeri* (Pr) are multidrug-resistant strains associated with nosocomial infections. The immune response against these pathogens had not been described previously. Our first study evaluated whether Ps and Pr triggered the bactericidal response in neutrophils (PMN). We reported that Ps and Pr were phagocytosed and induced some degree of respiratory burst. However, neither Ps nor Pr induced neutrophil extracellular traps (NETs) release. This lack of NETs formation may reflect an active strategy of immune evasion. Therefore, here our aim was to investigate the mechanisms behind the lack of NETs formation in the presence of Ps or Pr. We assessed whether Ps and Pr directly affect NET formation triggered by positive stimuli such as PMA and E. coli (Eco). Purified human PMN (n=6) were incubated with Eco (MOI 1) or PMA (40 nM) and Ps or Pr (MOI 10) for 3 h. We determined NETs by double-stranded DNA release and confocal microscopy. We found that NETs formation by Eco and PMA was inhibited by Ps or Pr ($p<0.05$). To determine whether this inhibition depends on bacterial viability or a soluble factor released from bacteria, we treated Ps and Pr with PFA (4%, 30 min) or added the supernatant of Ps or Pr cultures instead of bacteria to PMN and triggered NETs by PMA. The fixated bacteria did not affect PMA-induced NETs but the supernatants inhibited NETs triggered by PMA. Our results confirm that Ps and Pr are not only poor inducers of NET release, but that they inhibit NET induction triggered by positive stimuli. This inhibition is mediated by soluble factors released by the bacteria and is dependent on bacterial viability. In conclusion, Ps and Pr show active strategies to subvert PMN-mediated NET formation.

342. 154. **THE LACK OF IN VIVO OF NEUTROPHIL EXTRACELLULAR TRAPS FORMATION AFTER PROVIDENCIA SPP. INFECTION INCREASED BACTERIAL DISSEMINATION TO OTHER ORGANS**

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P. stuartii (Ps) and *P. rettgeri* (Pr) are clinically relevant opportunistic pathogens, causing a wide variety of nosocomial infections. The immune response against these strains is beginning to be studied. Neutrophils (PMN) are the first line of defense against bacteria, and have different microbicidal mechanisms to combat infections. The release of neutrophil extracellular traps (NETs) by PMN can trap microbes, preventing their spread to other tissues. We have previously reported that both Ps and Pr did not induce the formation of NETs in human PMN *in vitro* and showed an increased survival compared to an ATCC strain of *E. coli* (Eco), a high NETs inducer. Our aim was to study whether *Providencia* spp. induce NETs formation after infection in mice. We inoculated 10^7 bacteria to mice i.p. and after 24 h we performed peritoneal lavages (PL), and collected the spleen and lungs to study bacterial dissemination from the inoculation site. We used Eco for comparison. In the PL, we determined PMN counts, and measured double-strand (d.s.) DNA using Picogreen and myeloperoxidase (MPO) activity with a kit to evaluate the migration of PMN and NETs formation, respectively. We measured CFU in PL, spleen and lungs using TSA plates and used the ratio CFU in organs/CFU PL to account for bacterial dissemination (escape index). We found that although Ps, Pr and Eco caused a similar influx of PMN to PL, the levels of d.s. DNA and MPO activity were lower for Ps and Pr compared to Eco ($p<0.05$). The escape index of Ps and Pr for both spleen and lungs was higher compared to Eco ($p<0.05$). In conclusion, the lack of NETs formation at the inoculation site fails to contain Ps and Pr favoring their dissemination to other organs.

343. 165. **SARS-COV-2 AND ITS SPIKE PRO-**

TEIN INDUCES EXTRACELLULAR DNA RELEASE BY MAST CELLS

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Different pathogens activate mast cells to release DNA Extracellular Traps (DETs) composed of chromatin associated with granule proteins such as tryptase and LL-37. Activated mast cells also secrete pro-inflammatory cytokines and can induce vascular permeability, facilitating inflammation. This study hypothesizes that mast cell DETs induced by SARS-CoV-2 and its Spike protein are associated with the pathophysiology of COVID-19 and these DETs contribute to cellular damage. In the present work, we evaluated whether SARS-CoV-2 and its Spike protein could induce DETs release by mast cells. The human mast cell line HMC-1 was stimulated with different SARS-CoV- 2 MOIs and/or Spike protein concentrations (µg/mL) for 4 hours, and DETs were quantified using Picogreen and visualized by fluorescent microscopy. Statistical analyses were performed with GraphPad Prism 8.0.1 software. Our results showed DET production by HMC-1 at all different virus MOIs and at all Spike concentrations. We evaluated the signaling pathways involved in DET production, such as ROS, calcium, and serine protease activity, by pre-treating HMC-1 with inhibitors (NAC, DPI, BAPTA, and Nafamostat) prior to incubation with SARS-CoV-2 and/or Spike. DET production was measured after 4 hours. Our results showed that all inhibitors significantly reduced DET production, suggesting that DET release was dependent on ROS, calcium, and serine protease activity. We also evaluated the toxicity of DETs to A549 and Calu-3 lung cell lines by treating these cells with different concentrations of virus and Spike DETs (50, 100, 200, and 400 ng/mL) for 24 hours and measuring LDH release. We observed significant cell death in both cell lines treated with DETs, as indicated by LDH release. Our findings reveal that SARS-CoV-2 and Spike protein induces DET production by mast cells dependent on ROS, calcium, and serine protease activity, and these DETs are toxic to lung cells, possibly contributing to COVID-19 pathology.

344. 184. **GENERATION AND CHARACTERIZATION OF HUMAN INTESTINAL ORGANOIDS**

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Intestinal organoids are three-dimensional structures that mimic the cellular complexity and behaviour of the original intestinal tissue in vitro. These organoids, derived from crypt stem cells serve as valuable models for studying intestinal development, homeostasis, diseases, and regeneration. This study aimed to obtain and characterize human intestinal organoids from cells derived from different inflammatory intestinal samples. We analyzed intestinal biopsies (n=23) and surgical specimens (n=6) from adult patients with colorectal cancer (CRC), inflammatory bowel diseases (IBD) (n=19), and healthy controls (n=8), as well as colorectal polyps from pediatric patients sensitized to cow's milk protein (n=7) and biopsies of the surrounding areas (n=2). The frequency of Lgr5+ stem cells was carried out by flow cytometry. Organoid cultures were initiated by isolating intestinal crypts and embedding them

in Matrigel® with IntestiCult™ Organoid Growth Medium. These organoids were passaged every 7 or 10 days, differentiated with IntestiCult™ Organoid Differentiation Medium, and analyzed through fluorescence microscopy and real-time PCR. Our results showed an increased frequency of LGR5+ cells in inflamed IBD tissue compared to non-inflamed tissue and healthy controls (p=0.007). Conversely, fewer LGR5+ cells were found in polyp samples compared to control colonic biopsies (p=0.04). Organoids were successfully generated from various samples, and epithelial and stem cells were detected as Epcam+ or Lgr5+ cells by confocal microscopy. We also detected proliferating cells as Ki-67+ cells. In differentiated organoids, the transcripts corresponding to Lgr5 were diminished, while those coding for MUC2 and CHGA were increased compared to organoids developed in growing medium. In conclusion, we successfully generated and characterized human intestinal organoids from both healthy and inflammatory samples of adult and pediatric patients, and from intestinal pieces and biopsies. Further studies are needed to explore the functional role of epithelial cells in different inflammatory conditions.

345. 200. **NEUTROPHIL EXTRACELLULAR TRAPS (NETS) INDUCE MONOCYTE ACTIVATION THROUGH NF-KB SIGNALING PATHWAY**

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Background: 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, 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. **Objectives:** Thus, here we assessed if NETs activate monocytes, and analyzed the molecular pathways involved in the activation and functional modulation of these cells. **Methods:** Monocytes from healthy donors were treated with purified NETs (NET-containing supernatants centrifuged at 18K g) released by neutrophils exposed to inactivated Sars-CoV-2 (inCoV2) or HIV-1 (inHIV1). After 24 hours, cell culture supernatants were analyzed for production of reactive oxygen species (ROS) and inflammatory mediators through DHR probe and ELISA, respectively. **Results:** Monocytes treated with inCoV2-NETs released significant amounts of ROS, the pro-inflammatory cytokines IL-6 and TNF-α, and the β-chemokines MIP-1-α and RANTES. In addition, monocytes were also activated by inHIV1-NETs, producing higher levels of IL-6, TNF-α and β-chemokines. Since the transcription factor NF-kB is critical for inflammatory responses, we analyzed whether it was involved in NET-mediated monocyte activation. Thus, monocytes were treated with the NF-kB inhibitor BAY 11- 7082 30 minutes before cell exposure to inCoV2-NETs. We found that blocking NF-kB signaling reduced the production of the pro-inflammatory cytokines TNF-α and IL-6, and MIP-1α, suggesting that NETs activate monocytes through this molecular pathway. The compound BAY 11-7082 did not impact on monocyte viability. **Conclusion:** Taken together, our results show that interaction with NETs impact on 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

346. 201. **IMPROVING THE FUNCTIONAL PROPERTIES OF CIRCULATING NEUTROPHILS FROM IMMUNOCOMPROMISED MICE SUBJECTED TO INFECTIOUS STRESS BY IMMUNOBIOTICS**

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Polymorphonuclear neutrophils are the first line of defense against pathogens. The number of blood neutrophils depends on the balance between bone marrow (BM) granulopoiesis and apoptosis in peripheral tissues. Many current chemotherapeutic drugs have cytotoxic side effects that impair the life quality of the patients conditioning their treatment. We previously showed that both *Lacticaseibacillus rhamnosus* CRL1505 (Lr) and its cell wall (CW) were able to improve BM emergency myelopoiesis and protection against respiratory pathogens in mice undergoing chemotherapy. Here, we studied the functional properties of circulating neutrophils from immunocompromised mice fed with strain CRL1505 or its CW

subjected to infectious stress. Adult Swiss-mice were orally treated with Lr or CW during 16 consecutive days. On day 6, treated and untreated mice received one intraperitoneal dose of cyclophosphamide (Cy-150mg/kg). On day 9, mice were infected with *Streptococcus pneumoniae* (10^7 UFC/mice). The microbicidal activity, expression of integrins and formation of neutrophil extracellular traps (NETs) were evaluated after the pneumococcal challenge. The untreated group showed a decrease in the number of peroxidase+ cells in blood, a high expression of CXCR4 and a low expression of CD62L in Gr1+ cells of BM. The reduced neutrophil activation was consistent with reduced NETs formation in untreated mice. However, Lr and CW treatments were effective to significantly increase lung neutrophils and macrophages, blood neutrophils and peroxidase+ cells with respect to the untreated group. Besides, the treatment with CW was more effective than Lr to decrease retention signals in the BM cells. Furthermore, both treated groups showed a higher expression of SYTOX+ neutrophils compared to the untreated group, evidencing a recovery of the ability to form NETs. Therefore, Lr and CW improve the innate and myelopoietic response against *S. pneumoniae*, inducing an early recovery of the number, as well as a major microbicidal ability of circulating neutrophils.

347. 220. **ASSOCIATION BETWEEN MATERNAL OBESITY AND THE PHENOTYPE OF MONOCYTES AND HEMATOPOIETIC PROGENITOR CELLS IN THEIR OFFSPRING AT BIRTH**

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348. 230 **BACTERIALVIRULENCEFACTORYOPPMODULATESMURINEMACROPHAGEFUNCTIONSBYINTERACTIONWITHSURFACEN-GLYCANS**

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Background: *Yersinia enterocolitica* outer protein P (YopP) is a virulence factor that modulates early immuneresponse decreasing pro-inflammatory cytokines and nitric oxide (NO) production in macrophages(Mφs).TheparticipationofN-glycans inYopPeffectsonMφ are not clearlydefined. **Objectives:** We aimed to investigate whether the YopP immunomodulatory activity on Mφs is mediated bysurface N-glycans. **Methods:** MurineperitonealMφsweretreatedwithPNGaseFtoremoveN-glycansorwithalpha2,6sialidase(Neu) and infected with *Yersinia* wild type (Ye wt) or with *Yersinia* deficient in YopP (Ye Δ*yopP*). Wedetermined NO and inositol hexaphosphate (IP6) by colorimetric methods. The cells viability wasevaluated by LDH and MTT assays. Phagocytic activity and the expression of F4/80 and Ly6Cmoleculeswereanalyzedbyflowcytometry. StatisticalsignificancewasassessedbyANOVA,p≤ 0.05wasconsideredsignificant. **Results:** N-glycan removal did not impact Mφs functions except for decreased NO levels. Surprisingly, YopPexhibitedadualeffectonNOproduction:suppressioninnormalmacrophages($p<0.05$)butenhancement in N-glycan-deficient macrophages ($p<0.05$), implicating surface N-glycans in the NOregulationbyYopP.Toidentifyapotentialglyco-target,NOwasmeasuredafterNeutreatmentandYe wt infection. Neu-treated macrophages produced more NO than untreated controls ($p<0.05$). Inline,extracellularlactosetreatmentaugmentedNOininfectedmacrophages($p<0.05$).GiventheIP6dependence for YopP activity, IP6 production was evaluated. Thus, 30 min post-infection with Yewt,IP6productionwassignificantlyhigherthaninuninfectedMφs($p<0.001$).However,asignificantdecrease in IP6 levels was observed 45 minutes after

infection (p<0.0001). This decrease was not observed in infected Mφ treated with Neu. In contrast, Ye Δ*yopP* did not alter IP6 levels compared with uninfected controls. **Conclusion:** These results suggest that YopP influences macrophage NO and IP6 production via surface glycans.

349. 236. **GAMMADELTA T CELLS INTERNALIZE GLIOBLASTOMA CELL LINE-DERIVED EXTRACELLULAR VESICLES**

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Tumoral cells modulate their microenvironment by different means such as the release of extracellular vesicles (EVs). EVs interact with target cells by membrane receptors, by being internalized or by their fusion with plasma membranes. Then, molecules carried by EVs can be transferred to immune cells in the tumor microenvironment, modulating their functions. Gammadelta T cells are non-conventional T lymphocytes that recognize malignant cells and can trigger their apoptosis. Based on the anti-tumoral capacity of gammadelta T cells, immunotherapy protocols have been proposed to be employed to treat pathologies like Glioblastoma multiforme (GBM). GBM is the most aggressive malignant cerebral tumor in adults and has a median survival of less than a year after diagnosis. Previously, we demonstrated that GBM cell line-derived EVs interacted with gammadelta T cells (p<0.001). In this work, we aimed to study events that occur after EVs and gammadelta T cell interaction. For that purpose, peripheral blood gammadelta T lymphocytes were purified by using an anti-TCR gammadelta MicroBead isolation kit and EVs were obtained from the U251 cell line by differential centrifugation. First, gammadelta T cells stimulated or not with the specific agonist HMBPP, were incubated overnight with different EVs quantities. Then, T cell activation was analyzed by measuring CD69 expression by flow cytometry. These studies showed that EVs obtained from 6x106 or 8x106 U251 cells upregulate CD69 expression in non-stimulated or HMBPP-stimulated gammadelta T cells, respectively (p<0.05). Furthermore, EVs were stained with the lipophilic fluorescent dye PKH26, incubated with gammadelta T cells and used to perform time lapse assays by confocal microscopy. Interestingly, the 3D analysis showed that EVs were inside gammadelta T cells, and that was more noticeable when T cells were pre-activated with HMBPP. Our findings suggest that gammadelta T lymphocytes may internalize GBM-derived EVs and be activated by them.

350. 242. **MODULATION OF MURINE DENDRITIC CELL-DERIVED EXTRACELLULAR VESICLES BY THYROID HORMONE TRIIODOTHYRONINE (T3): IMPLICATIONS ON EXTRACELLULAR VESICLES RELEASE AND FUNCTION**

Mariana Pires Teixeira[1], Dana Negretti-Borga[1], Vanessa Sandim[2], Brunno Renato Farias Verçoza[3], Antonela Blanco[1], Elida Nahir Puentes[1], Russolina Benedeta Zingali[2], Ana Carolina Donadio[1], María Del Mar Montesinos[1], Robson de Queiroz Monteiro[2], Claudia Gabriela Pellizas[1] 1. Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI- CONICET), Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Bioquímica Clínica, Córdoba, Argentina. 2. Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. 3. Núcleo Multidisciplinar de Pesquisa UFRJ Xerém (NUMPEX-BIO), Universidade Federal do Rio de Janeiro - Campus Duque de Caxias, Rio de Janeiro, Brazil. Dendritic cells (DCs) secrete extracellular vesicles (EVs) that modulate immune responses. Our group has demonstrated that T3 activates DCs, promoting Th1 and Th17 adaptive responses, as well as antigen-specific cytotoxic responses in vitro and in vivo. Nevertheless, T3 effects on DCs-derived EVs are still unknown. We aimed to quantify and characterize EVs from T3-treated DCs and investigate their impact on immune responses. Bone marrow cells from C57BL/6 mice were differentiated into DCs using GM-CSF and then cultured with 10 Mm of T3 (DC-T3) or without (DCs-Ct) for 18h. Conditioned medium (CM) was centrifuged to remove cells/debris (300g 10´/2000g 20´), and to obtain 10K-EVs (10000g 40´) and 100K-EVs (100000g 90´). EVs quantification was performed using NTA and Bradford, and characterization

using WB. EVs from DCs-Ct and DCs-T3 were co-cultured with syngeneic DCs, for the analysis of EV uptake and DCs activation markers (MHCII and CD86) expression, or with Balb/c mice splenocytes, for the analysis of T cell activation marker (CD25) expression by FACs. For statistical analyses p≤0,05 were considered significant. A significant increase of 10K-EVs concentration and a tendency of increase of 100K-EVs was found in DCs-T3 (vs DCs-Ct) by NTA (paired t-test), though not by Bradford. WB showed increased expression of exosomes markers and decreased microvesicles marker in 100K-EVs (vs 10K-EVs). Preliminary results using CFSE-labeled EVs suggest uptake of both EVs by DCs. DCs-T3-derived EVs and CM of DCs-T3 (EVs-depleted), but not DCs-Ct-derived EVs nor CM of DCs-Ct, significantly increased %CD86high DCs (ANOVA- Tukey test). Additionally, preliminary results showed no significant differences in $\%CD3^{+}CD25^{+}$ cells when splenocytes were cultured with either EVs or CM, but a tendency of increase in $\%CD3^{+}CD8^{+}CD25^{+}$ cells was observed when splenocytes were cultured with DCs-Ct-derived EVs (vs splenocytes). In conclusion, T3 increases EV release by DCs and appears to affect their function, potentially impacting immune responses.

351. 245. EFFECT OF AGARICUS BISPORUS EXTRACT AND FRACTIONS ON MURINE MACROPHAGES

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Medicinal mushrooms are important sources of natural bioactive compounds with therapeutic applications. Among them, those presenting immunomodulatory activity stand out, such as proteins. The objective of this research work was to study the biological effect of *Agaricus bisporus* (*Ab*) extract and three fractions of different molecular weight, on murine macrophage cell line RAW 264.7. To achieve this goal, fruiting bodies of *Ab* were lyophilized and homogenized with Tris-Glycine buffer pH 8.4. Homogenates were centrifuged and supernatants were treated with ice cold ethanol to precipitate soluble proteins. The precipitates were resuspended and the extract was obtained. In order to separate the fractions, centrifuge filtration tubes were used (pore size 30 and 100 kDa), obtaining three fractions (<30 kDa, 30-100 kDa, >100 kDa). Proteins, polyphenols and reducing sugars concentrations of *Ab* extract and fractions were determined. To assess cellular metabolic activity, RAW 264.7 cells (150.000 cells/well) were seeded in 24-well plates and were incubated at 37°C with different protein concentration of *Ab* extract or fractions for 24 h. Supernatants were collected and stored at -20°C, and cells were incubated with MTT for 30 min to carry out MTT assay. For nitrites quantification, Griess assay was performed using the previously collected supernatants. T-test and ANOVA statistical analyses with Dunnett's *post-test* were performed. The results obtained showed that *Ab* extract induces the activation of murine macrophages at a cytotoxic concentration. On the other hand, it was observed that the smaller molecular size fraction promotes greater activation in cells compared to the extract, while higher molecular weight fractions generate dose-dependent activation of murine macrophages, without altering cell viability. These results show the potential of the fractions obtained as possible immunostimulators to be applied in the treatment of various pathologies or as adjuvants in vaccines.

352. 249. N-ACETYLCYSTEINE PROMOTES DIFFERENTIATION OF MONOCYTES INTO DENDRITIC CELLS

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Monocytes are highly plastic cells able to differentiate into macrophages (Mo-Macs) or DCs (Mo-DCs), however, the factors and the mechanisms that govern this cell-fate decision remain poorly characterized. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that induces the differentiation of monocytes into Mo-Macs, while the combination of GM-CSF plus IL-4 is widely used to generate Mo-DCs. The role of reactive oxygen species has been studied in the context of macrophage M1/M2 polarization, however, its role in the differentiation of monocytes into Mo-Macs vs Mo-DCs has not been defined. Here, we show that the potent antioxidant N-acetylcysteine (NAC) modified the biological activity of GM-CSF from a macrophage differentiation factor toward a DC differentiation factor. Human monocytes were isolated from healthy do-

nors by conventional methods and incubated with GM-CSF (50 ng/ml) or GM-CSF (50 ng/ml) plus NAC (5 mM) for 6 days, and the expression of CD1a and CD16 was analyzed by flow cytometry. Mo-DCs was characterized as CD1a+CD16- cells while macrophage-like cells were characterized as CD1a-CD16+ cells. We found that NAC markedly promoted Mo-DC differentiation in GM-CSF treated monocytes: %CD1a+CD16- cells 18.1 ± 2.2 vs 49.8 ± 3.2 for GM-CSF vs GM-CSF + NAC (n=24 p<0.0001). As expected, treatment with LPS (10 ng/ml) for 18 hs significantly (p<0.05) increased the expression of CD86 in Mo-DCs differentiated by treatment with GM-CSF plus NAC: increase in the mean fluorescence intensity 289 ± 32% (mean ± ES, n=6). No significant increase in CD86 expression was observed for monocytes treated only with GM-CSF. Interestingly, Alk5, an inhibitor of the TGF-β receptor completely prevented the ability of NAC to promote the differentiation of Mo-DCs from GM-CSF-treated monocytes; % inhibition higher than 90%, n=3). Our results uncover a new pathway able to induce the differentiation of Mo-DCs.

353. 254. **IMPACT OF EGC OPERON SUPERANTIGENS SEI, SEO, SEG, AND SEM ON NEUTROPHIL ACTIVATION**

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Superantigens (SAgs) are enterotoxins that promote massive cytokine release and immunosuppression and primarily produced by extracellular bacteria, such as *S. aureus*. Polymorphonuclear cells (PMNs), mainly neutrophils, are the first line of defense against these pathogens, being a key component of the innate immune system. Here, we investigated the effect of four natural SAgs from the *egc* operon (SEG, SEI, SEO and SEM) on innate immunity, focusing on neutrophils. For this purpose, SEI, SEO, SEG and SEM were cloned and produced as recombinant proteins in *E. coli* and purified by Ni^{++}/NTA column. PMNs were isolated from healthy donors and incubated with 1 μM of each SAg or with PMA as a positive control, at various time points (0.5, 4, and 24 hours) depending on the assay. The neutrophil respiratory burst was evaluated by flow cytometry, using dihydro-rhodamine 123 (DHR). NETs release was evaluated by IFI. Supernatants were collected for quantitative analysis of DNA using Sytox Green, and measurement of IL-1β, IL-6, IL-8, IL-12 and TNF-α by ELISA. Results were analyzed by one and two-way ANOVA with the corresponding post-hoc test. PMNs stimulated with 1 μM of SEO and SEM produced a significative increase in rhodamine fluorescence (p<0.05), indicative of early activation. *Egc* SAgs also induced the release of NETs, visualized and quantified by confocal microscopy and by released free DNA (p<0.05). Additionally, all SAgs induced a higher production of IL-1 β, IL-6, IL-8, IL-12 and TNF-α by PMNs compared to untreated cells (p<0.0001). In conclusion, these results show that the SAgs SEI, SEO, SEG and SEM induced the release of NETs from PMNs with associated production of pro-inflammatory cytokines, IL-1β, IL-6, IL-8, IL- 12 and TNF-α. The effects of *egc* SAgs on PMNs underscore their ability to influence not only the adaptive immune response but also the innate immune system.

354. 257. **CYTOTOXICITY AND MODULATION OF IL-10 AND TNF-ALPHA PRODUCTION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS, OF NANOPARTICLES WITH ARACHIS HYPOGAEA L. TEGUMENT EXTRACT CAMILA**

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In viral infections, cytokines production is deregulated, contributing to immunopathogenesis. Plant extracts could stimulate the immune system. These compounds are often degraded before consumption. Their incorporation into nanoparticles increases their intestinal absorption and bioavailability. The aim was to evaluate *in vitro* cytotoxicity and production of IL-10 and TNF-α of nanoparticles with *Arachis hypogaea* L. tegument ethanolic extract (TEE).
Soy lecithin multilamellar vesicles with TEE were obtained and subjected to extrusion resulting in unilamellar vesicles with TEE (UVE). Then, they were run into a sephadex column to obtain purified unilamellar vesicles (PUV). Empty unilamellar vesicles (EUV) without TEE were produced. Cytotoxicity and immunomodulation studies were performed with peripheral blood mononuclear cells extracted from healthy humans (Banco de Sangre, UNC) using Ficoll-Hypaque. Cells were seeded in 96-well plates with RPMI medium supplemented and treated with UVE, PUV (50-200µg/mL of TEE) and EUV, for 48 hours. Cell viability was assessed by trypan blue exclusion. To assess cytokine production, PBMCs were stimulated with dengue virus serotype 2 (DENV2), and were treated with UVE, PUV (50µg/mL of TEE) and EUV (10%). Cell controls (CC) and positive control (VC), treated with DENV2 (50 PFU/well), were included. TNF-□ and IL-10 production was assessed in supernatants by commercial ELISA kits. ANOVA, t-test, and nonlinear regression analysis were performed (GraphPadPrism 8.0). UVE, PUV, and EUV were not cytotoxic. TNF-α production was 168.5±45.25 and 131.1±4.71pg/mL for UVE and PUV, respectively with a significant difference to VC (498.1±64.58pg/mL). IL-10 production also showed significant differences between UVE (724.7±57.66pg/mL) and PUV (718.9±13.60pg/mL) and VC (1243±151.8pg/mL) . No significant differences were found for EUV in any case. These results demonstrate that nanoparticles with TEE could modulate immune response to exposure of viral infections at non-cytotoxic concentrations

355. 271. **EXPLORING THE IN VIVO IMPACTS OF EXOGENOUS CD40 AGONISTS AND CONSTITUTIVE ENDOGENOUS CD40L ON PERITONEAL CAVITY MACROPHAGE RESPONSES TO IL-4**

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Type 2 immunity is mainly driven by the cytokines IL-4 and IL-13, which cause macrophages to expand through proliferation and to express M(IL-4) markers including RELM-α and Chil3 (Ym1). The CD40-CD40L interaction has central roles in immunology, including inducing classical macrophage activation. However, little is known about its role in the Th2 side of immunology. In the inductive arm, although strong exogenous CD40 agonists oppose Th2 responses, CD40 and CD40L are necessary for the proper development of these responses. This may be explained by CD40 agonists generating different signals depending on format and dose. We were particularly interested in possible effects of constitutive endogenous CD40L. We detected cell-surface CD40L in the peritoneal cavity under basal conditions only in $CD4^+$ T cells, mostly naïve cells, similarly to what was previously reported for spleen. Also similar to those previous reports, the abundance of cell-surface CD40L was enhanced in CD40-deficient animals, reflecting negative regulation of CD40L exposure through interactions with CD40-expressing cells. IL-4 injection caused similar levels of macrophage proliferation and expression of RELM- α and Ym1 in CD40-deficient and WT mice. In addition, CD40 KO or WT peritoneal macrophages transferred into the peritoneal cavity of WT mice proliferated and up- regulated RELM-α and Ym1 similarly. Also, CD40L-blocking antibody had minimal inhibitory effect on peritoneal macrophage proliferation and did not affect M(IL-4) marker expression upon IL-4 injection. On the other hand, exogenous activation of CD40 by an agonistic antibody (1C10) significantly blunted

the responses of peritoneal macrophages to exogenous IL-4 in terms of proliferation and expression of M(IL-4) markers. In sum, a strong exogenous CD40 agonists act as negative regulators of type 2-associated macrophage responses, whereas any basal interactions between macrophage CD40 and constitutive CD40L in $CD4^+$ T cells do not significantly impact on the IL-4-driven macrophage responses in the peritoneal cavity.

356. 281. **CD40L-CD40 INTERACTION: AN IN VITRO EXPLORATION OF ITS IMPACT ON MACROPHAGE POLARIZATION IN RESPONSE TO IL-4**

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The CD40 ligand (CD40L) - CD40 interaction plays crucial roles in diverse immunological processes. CD40L is mostly expressed by activated $CD4^+$ T cells, and macrophages express its main receptor CD40. Whereas the role of this interaction in classical macrophage activation is well-characterized, its impact on M(IL-4) macrophage polarization typical of Th2 contexts is less understood. This study explores this issue using resident peritoneal cavity macrophages in culture. Mouse peritoneal cavity cells or macrophages purified from these were stimulated with IL-4 alongside recombinant soluble CD40L (sCD40L) or 1C10 (a CD40 agonist antibody). The agonists caused, as expected, up-regulation of MHC-II and CD80, usually considered classical activation markers. Both CD40 agonists strongly inhibited the expression of M(IL-4) marker RELM-α, but had minimal effects on a second marker, ChiL-3 (Ym1). To explore whether these effects occur in the context of antigen-specific interactions, we co- cultured splenocytes from OT-II mice with purified peritoneal macrophages from WT or $CD40^{-/-}$ mice pre-loaded or not with OVA peptide, adding or not IL-4. Surface CD40L expression was induced in the $CD4^+$ T cells in response to antigen presentation as expected. The induction was stronger in co-cultures with $CD40^{-/-}$ macrophages, showing that surface CD40L can be regulated by CD40 in peritoneal macrophages, similar to what was reported for other CD40-expressing cell types. Also as expected, antigen presentation upregulated MHC-II on macrophages, more strongly on WT than $CD40^{-/-}$ cells. RELM-α expression was inhibited by antigen presentation, with a strong trend towards deeper inhibition in WT than in $CD40^{-/-}$ macrophages. Ym-1 was only slightly impacted in both genotypes. Our preliminary conclusion is that the CD40L-CD40 interaction inhibits RELM-α expression but affects Ym1 weakly. This suggests that CD40 engagement in macrophages selectively alters events in the PI3K/AKT-dependent branch of IL-4 receptor signaling, known to be necessary for the expression of RELM-α but not Ym1.

357. 292. **NEUTROPHIL EXTRACELLULAR TRAPS PROMOTE MACROPHAGE ACTIVATION THROUGH TLR-2 AND NF-KB SIGNALING PATHWAYS**

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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 and myeloperoxidase. These traps are released upon neutrophils' contact with several factors, including inflammatory mediators and infectious agents. Since we recently published that NETs control

HIV-1 infection in macrophages, and to better understand the impact of NET-macrophage interaction in cellular activation, we are currently investigating macrophage responses to these structures in a non-infectious context. Monocyte-derived macrophages from healthy donors, obtained by density gradient centrifugation and adherence onto plastic plates, were exposed to NETs induced by inactivated SARS-CoV-2 (inCoV-2) or inactivated HIV-1 (inHIV-1). In some assays, pharmacological inhibitors were used. ELISA method was used to quantify inflammatory mediators after 24 hours in culture supernatants and to measure NF-κB phosphorylation in cell lysates after 2 and 8 hours. NETs increased macrophage production of reactive oxygen species and promoted NF-κB activation. Furthermore, NETs induced the release of the β-chemokines RANTES, MIP-1α and MIP-1β, as well as IL-6, IL-8 and TNF-α. To investigate receptors engaged in NET recognition and cellular activation, we used inhibitors of Toll-like receptor (TLR) 2 and 4. The inhibition of TLR2 and NF-κB downstream signaling abrogated macrophage production of inflammatory mediators induced by NETs. RNA sequencing revealed that more than thirty genes were differentially expressed, and sixteen biological processes and two molecular functions were enriched in NET-treated macrophages. Proteomic analysis of NETs released upon neutrophil activation by four different stimuli (*Leishmania,* inCoV2, inHIV-1 and IL-8) detected ~2000 proteins, including those more frequently described associated to chromatin, such as neutrophil elastase, myeloperoxidase, cathepsin, HMGB1, histones and S100 family. These results indicate that macrophage transcriptional profile and functioning is modulated upon interaction with NETs through TLR-2 and NF-kB signaling pathways.

358. 296. **PIMOZIDE BOOSTS CROSS-PRESENTATION AND CD8+ T CELL PRIMING BY DENDRITIC CELLS**

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Dendritic cells (DCs) are key players in initiating and regulating adaptive immune responses by capturing, processing, and presenting antigens to CD8+ T cells via cross-presentation (XP). Understanding the factors that influence XP in DCs is crucial for developing effective immunotherapies. Our current research aims to identify new adjuvants that enhance CD8+ cytotoxic T lymphocyte (CTL) responses. Through high-throughput screening (HTS), we identified 5 compounds and among them we selected Pimozide (P) as a promising compound that could enhance XP. Our findings demonstrate that P significantly enhances XP in DCs, leading to improved priming of CD8+ T cells in vitro. This effect is associated with increased translocation of antigens into the cytosol, resulting in greater expression of the H2Kb-SIINFEKL complex on the DC surface. Additionally, P treatment induces the production of reactive oxygen species (ROS). Preliminary data suggest that P might modulate lipid peroxidation, a process closely linked to ROS production. We hypothesize that this could compromise endosomal membrane integrity, facilitating antigen escape into the cytosol, a critical step in XP. Moreover, ROS generation might also affect endosomal acidification, potentially altering the pH and influencing antigen degradation, thereby preserving antigenic fragments suitable for XP. Ongoing studies aim to further elucidate the impact of Pimozide on endosomal pH and clarify the specific role of ROS in modulating this process. Lastly, *in vivo* experiments demonstrate that vaccination with P-stimulated DCs induces a robust CTL response, highlighting the potential of Pimozide as an adjuvant in DC-based immunotherapies targeting CD8+ T cell-mediated immunity. These findings suggest that Pimozide could be a valuable tool in developing more effective immunotherapies against infections and cancer. Further research will clarify the molecular targets activated by Pimozide in DCs and its potential as a novel adjuvant.

359. 297. **EXPRESSION OF SLAMF1 AND PD-L1 IN NEUTROPHILS FROM PATIENTS WITH ACTIVE TUBERCULOSIS CORRELATES WITH THE SEVERITY OF THE DISEASE**

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Background: Neutrophils are the first line of defense against pathogens, combating them by using several antimicrobial mechanisms such as phagocytosis, reactive oxygen species and neutrophil extracellular traps, being the predominantly infected cells in the human lungs during active *Mycobacterium tuberculosis (Mtb)* infection. We previously demonstrated that SLAMF1 activation in *Mtb*-Ag stimulated neutrophils significantly augmented intracellular ROS levels. Here we further analyzed surface molecules´ expression and the microbicidal capacity of neutrophils from tuberculosis (TB) patients presenting distinct immunological status and disease severity. **Objective:** To elucidate if the differential expression of the surface molecules SLAMF1 and PD-L1 correlates with microbicidal ability of neutrophils in TB patients with different disease severity. **Methods:** TB patients were classified as high or low responders (HR or LR) in accordance with their T cell activity against a *Mtb* lysate (*Mtb*-Ag). Neutrophils were purified from heparinized blood by Ficoll-Hypaque centrifugation, dextran sedimentation, and hypotonic lysis. Cells (2×10^6/mL) were stimulated with *Mtb*-Ag for 2h and stained with anti-SLAMF1 and anti-PD-L1 fluorophore-conjugated antibodies. For Colony Forming Unit (CFU) assay neutrophils ($2.5x10^6$/mL) were infected with *Mtb* H37Rv (MOI:5) for 2h, incubated for 24h, washed and lysed. CFUs were counted on Middlebrook 7H11 agar plates. **Results:** We found that, in comparison with LR TB patients, HR TB patients showed a significantly higher percentage of $SLAMF1^+$ and $PD\text{-}L1^+$ neutrophils after *Mtb*-Ag stimulation (Mann-Whitney test $p<0,0001$;$p<0,01$). Interestingly, neutrophils from both groups of TB patients phagocytized *Mtb* H37Rv strain equally. However, HR TB´s neutrophils displayed a significantly increased ability to kill pathogenic *Mtb* as compared to neutrophils from LR TB patients (unpaired t-test $p<0,05$). **Conclusion:** Our present results suggest that modulation of SLAMF1 and PD-L1 levels in neutrophils from TB patients with weak immune response against *Mtb* might be a promising target for TB host-directed therapy.

360. 311. THE ROLE OF BETA-CATENIN ACTIVATION IN MODULATING INNATE CD8+ T CELL DIFFERENTIATION DURING ACUTE T. CRUZI INFECTION

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During the acute stage of Th1 infectious processes, as *T. cruzi* infection, CD8+ thymocytes alter their differentiation from “conventional” to “innate” lineage. Innate CD8+ thymocytes (TIM) express a particular phenotype (CD44hi, CD122hi, EOMEShi) and participates in early protection. The Wnt/Beta-catenin signaling pathway assumes a crucial regulatory function in infectious and inflammatory processes. We have reported the activation of b-cat in both spleen cells, thymus and macrophages following *in vivo* and *in vitro T. cruzi* infection. In addition, we have reported that b-cat activation promotes TIM differentiation. To evaluate, using multiparametric flow cytometry (FACS), the role of *T. cruzi* infection and beta-catenin activation on the modulation of TIM cells using *in vivo* and *in vitro* experimental models. Thymocytes from 12-day B6-infected mice were stained for phenotyping by FACS The data were processed using R and FlowJo. For *in vitro* cultures, thymocytes from OT-I mice (targets) were cultured with thymocytes from WT or *T. cruzi*-infected mice (effectors) in the presence or absence of β-catenin activators (BIO) or inhibitors (iCRT14), or vehicle. After 48 hours, the degree of differentiation of target cells (OVA tetramer+) into TIM cells was analyzed by FACS. Twelve clusters based on CD44, CD122, EOMES, CD49d, beta-catenin, CD73, and CD124 were identified and visualized using UMAP. Cluster called 1, expressing the most TIM markers and IL-4 receptor (CD124), was significantly increased by the infection and showed increased beta-catenin expression. BIO treatment raised CD44 expression (MFI) and the frequency of CD122+, Eomes+, and CD124+ cells in the cultures. Conversely, the b-cat inhibitor iCRT14 decreased both the frequency and MFI of CD44+, Eomes+, CD122+, and CD124+ thymocytes in

the cultures. *T. cruzi* infection promotes the development of TIM cells, with b-cat playing an essential role in this process.

361. 316. **THE TAM AXIS AND MTOR PATHWAY GENES ARE DIFFERENTIALLY EXPRESSED IN CIRCULATING MONOCYTES OF PATIENTS WITH DIFFERENT HISTIOCYTIC DISORDERS**

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Background. The term “histiocytic disorders” groups a heterogeneous set of diseases characterized by the accumulation of histiocytes (mononuclear phagocytes derived from CD34+ stem cells or monocyte precursors) in different tissues. It includes Juvenile Xanthogranuloma (XGJ), Erdheim-Chester (ECD), Langerhans cells histiocytosis (LCH), and Hemophagocytic Lymphohistiocytosis (HLH). To this day, the knowledge of the triggers of these pathologies is scarce, thus making diagnosis and treatment challenging. **Objective.** Here, we aim to preliminarily compare the transcriptional program of the TAM tyrosine kinase axis and the mTOR pathway in CD14 monocytes to discriminate each entity better. **Methods.** Quantitative PCR (qPCR) analysis was performed in CD14 monocytes isolated from peripheral blood mononuclear cells (PBMCs) of healthy donors (n=5), patients with osseous LCH (n=5), ECD (n=2), XGJ (n=2) and HLH (n=3), targeting AXL, TYRO3, MERTK, GAS6, PROS1, LXR, KLF4, IRF4, FOXO1, SKGB1, EEIF4B, TNFα and IL-17. Healthy controls comprised male and female adults. LCH, XGJ, and HLH samples were obtained from children aged 1-8 years, both male and female. The ECD samples were from a 15-year-old girl and a 40-year-old male. **Results.** These preliminary results show that the TAM elements are differentially expressed. Higher mRNA levels of MERTK, AXL, GAS6, and PROS1 were found in CD14 monocytes of patients with HLH, while TYRO3 and PROS1 were more expressed in bone LCH. The onco-lymphoid IRF4 gene was highly expressed in HLH, with the lowest level being LCH. FOXO1 showed an increased trend for LCH and KLF4 for ECD. Finally, higher levels of inflammatory cytokines TNFα and IL-17 were found in XGJ patients. **Conclusion.** The observed differential gene expression patterns in CD14 monocytes associated with specific histiocytic disorders stimulate further investigation in a larger patient cohort to identify key genes for improved diagnosis and treatment.

362. 317. **INDOMETHACIN REDUCED THE EXPRESSION OF BONE-HOMING MOLECULES IN MONOCYTES OF PATIENTS WITH OSSEOUS LANGERHANS CELL HISTIOCYTOSIS**

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Background. Langerhans Cell Histiocytosis (LCH) is a rare and chronic disease frequently seen in children. When the osseous system is compromised, indomethacin, a nonsteroidal anti-inflammatory drug, has proven to be an effective treatment in these patients. Despite that, the particular mechanism of action of indomethacin in this disease has not been established. We have previously seen in-vitro that indomethacin affects the expression of bone-related chemokines and migratory receptors in monocytes, dendritic cells, and Langerhans-like cells. Therefore, we hypothesize indomethacin may be affecting target molecules involved in homing pathological monocytic precursors to the bone and receptors that participate in bone homeostasis. **Objectives.** Compare and correlate the expression of homing and ho-

meostatic bone genes in monocytes isolated from the blood of osseous LCH patients treated or not with indomethacin versus healthy controls (HC). **Methods.** CD14+ monocytes were isolated from peripheral blood mononuclear cells of patients with osseous LCH, both with (n=9) and without (n=16) indomethacin treatment, as well as from healthy controls (HC) (n=7). The samples included children and adults aged 2 to 50 years, with 68% of the participants being male. HC consisted of adult volunteers. Gene expression was analyzed using qPCR. **Results.** Circulating CD14+ monocytes of patients with bone LCH expressed higher mRNA levels of AXL, TYRO3, MERTK, PROS1, GAS6, CCR1, RANK, CD137, and OPG than HC. Most patients under indomethacin treatment showed lower AXL, GAS6, TYRO3, PROS1, RANK, OPG, and CXCR4 mRNA levels. **Conclusions.** Treatment with indomethacin reduced the expression of bone-homing and homeostasis molecules in monocytes of patients with osseous LCH, suggesting a possible impact on the migration of pathological precursors. Further longitudinal studies are needed to strengthen the role of indomethacin in cell migration and its potential benefits for patients.

363. 328. **NEUTROPHIL EXTRACELLULAR VESICLES MODULATE NEUTROPHILS RESPONSE TO LEISHMANIA PARASITES**

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In Leishmania infection, neutrophils are the first cells to migrate to the infected 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 neutrophil's role 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 form 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 towards this chemokine. In vivo, SEVs worsened ear lesions caused by Leishmania infection and increased parasite load as well. Ou results show that EVs released by neutrophils modulate neutrophil's function, impacting in vivo infection by Leishmania parasites. Future studies will focus on characterizing the vesicle content and exploring the complex mechanisms by which they influence infection outcomes.

364. 350. **NEUTROPHILS FROM B-1 CELL DEFICIENT MICE SHOW INCREASED MICROBICIDAL ACTIVITY**

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Neutrophils are crucial cells from innate immunity that act as the first line of defense against pathogens and/or tissue injury. They migrate to sites of infection or inflammation in response to chemical signals released by other cells. They perform effector mechanisms such as phagocytosis, degranulation and emission of neutrophil extracellular networks (NETs). Furthermore, they interact with other cells of the immune system, influencing the activation and differentiation of T and B cells. B-1 cells, a subtype of B lymphocytes that produce natural antibodies, can modulate the inflammatory response through the production of IL-10 cytokine. In this work we analyzed the in-

fluence of B-1 cells on the functional activity of neutrophils, using as a model XID mice deficient in B-1 cells. Analysis of our results by one-way ANOVA followed by Dunnett's post-test revealed that XID mice have a decrease 25% in peripheral blood neutrophils when compared to BALB/c mice. However, in the peritoneal cavity, while BALB/c mice have 15% neutrophils, XID mice have 30%. Furthermore, neutrophils from XID mice showed significant increased expression of MHCII molecules when compared to neutrophils from BALB/c mice. Additionally, we observed that neutrophils from XID mice have a high phagocytic activity (100%) when compared to neutrophils from BALB/c mice (50%). The data also showed that neutrophils from XID mice produce higher levels of NO and ROS molecules involved in increasing microbicidal activity. Finally, we demonstrated that neutrophils from XID mice release more NETs when compared to neutrophils from BALB/c mice. Based on our results, we suggest that in the absence of B-1 cells and their immunomodulatory action, neutrophils from XID mice exhibit increased microbicidal activity that may contribute to a pro-inflammatory profile.

365. 356. **ENGINEERING MICROGLIAL RECEPTORS TO IMPROVE IMMUNORESILIENCE IN ALZHEIMER'S DISEASE**

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Pharmacological activation of receptors on Disease Associated Microglia (DAM), such as TREM2, is emerging as a potential disease-modifying therapy in Alzheimer's Disease (AD). Nevertheless, the specific microglial mechanisms that safeguard against AD disease progression are yet to be fully understood. Here we show that sustaining physiological activation of the TAM family tyrosine kinase AXL correlates with protection against AD-associated memory loss in a mouse model. Conversely, absence of AXL in microglia exacerbated/accelerated cognitive decline. The disease-modifying effect of AXL augmentation correlates with a specific transcriptional state in microglia that is distinct from and independent of TREM2, and with an immuneresilient state that maintains tissue homeostasis despite chronic insult.

366. 357. **INHIBITION OF CD4+ T CELL PROLIFERATION BY IL-12 AND IL-18 PRE-ACTIVATED NK CELLS**

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Natural Killer (NK) cells are key effectors against tumors and virus-infected cells. Cytokine-activated NK cells show enhanced anti-tumor and antiviral activity. However, it has been shown that in different settings NK cells can inhibit T cell responses. Our previous work demonstrated that tumor-experienced NK cells express high levels of PD- L1, and inhibit $CD8^+$ T cell proliferation through PD-L1. In this study, we aimed to investigate whether cytokine-activated NK cells can regulate T cell proliferation. To this end, NK cells isolated from healthy donors were stimulated with IL-12 and IL-18 for 24 hours. CFSE-labeled peripheral blood mononuclear cells were stimulated with anti-CD3 to induce proliferation, and co-cultured with increasing numbers of either control or cytokine-stimulated autologous NK cells. After 5 days, T cell proliferation was assessed by flow cytometry as CFSE dilution. Our results showed that $CD4^+$ T cell-proliferation was reduced in a dose-dependent manner in the presence of cytokine-activated NK cells compared to control NK cells ($p<0.01$), while $CD8^+$ T cell proliferation and T cell viability was unaffected. Neutralization of the immunosuppressive cytokines IL-10 and TGF-β did not restore $CD4^+$ T cell proliferation. Furthermore, supernatants from cytokine-stimulated NK cells did not inhibit T cell proliferation, suggesting that the observed suppression is not mediated by soluble factors. Increased expression of PD-L1 was observed on cytokine-stimulated NK cells ($p<0.001$). However, inhibition of $CD4^+$ T cell proliferation persisted even when co-cultured with blocking antibodies against PD-1 or PD-L1. In conclusion, cytokine-stimulated NK cells express PD-L1 and can inhibit $CD4^+$ T cell proliferation; however this inhibition is not mediated through PD-L1 interaction or immunosuppressive cytokines. Further research is needed to

elucidate the mechanisms underlying this inhibition, which should be considered when designing therapeutic strategies involving the pre-activation of NK cells with cytokines.

367. 394. **SEMINAL PLASMA ATTENUATES NEUTROPHIL FUNCTIONALITY: THE ROLE OF PROSTAGLANDIN E2**

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Semen is composed for various cell types and soluble mediators, including cytokines and metabolites. It is well established that semen can induce both inflammatory and anti-inflammatory responses in the female genital tract. Neutrophils are the first cell type to reach the vaginal lumen following semen deposition, contributing to immunological clearance through the phagocytosis of sperm cells. However, the specific immune phenotype that neutrophils acquire upon exposure to soluble immune mediators in seminal fluid remains unclear. In this study, we investigate the ability of neutrophils to be activated over exposure periods to seminal plasma (SP) and the subsequent implications for their functionality. Semen samples from healthy donors were used to obtain SP and spermatozoa, which were purified through density gradient centrifugation. Neutrophils were isolated from blood by Ficoll-Paque centrifugation followed by dextran sedimentation. Neutrophil phenotypes were analyzed using flow cytometry. The interaction between neutrophils and sperm cells resulted in the upregulation of CD11b ($p=0.043$, $n=5$) and CD66 ($p=0.037$, $n=5$). However, this effect was diminished in the presence of SP. Furthermore, neutrophils incubated with SP exhibited a significant reduction in ROS production following 15 minutes of exposure to SP and fMLP ($p=0.0008$, $n=8$). Notably, this reduction was also observed when prostaglandin E2 (10^-6M), an abundant inhibitor of neutrophil function in SP, was added ($p=0,0295$, $n=4$). Lastly, we aimed to determine whether SP induces an anti-inflammatory profile in neutrophils. To this end, neutrophils were preincubated 15 minutes with SP, washed, and then activated with fMLP. This pre-exposure to SP was sufficient to reduce the ability of neutrophils to produce ROS. In summary, these findings indicate that SP can attenuate the activation of neutrophil cells by inhibiting ROS production and degranulation. This suggests a novel immunoregulatory function of SP.

368. 396. **TRANSCRIPTOMIC PROFILING OF HIGH SALT STIMULATED NEUTROPHILS**

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High salt (NaCl) concentrations are found in a variety of tissues under physiological and pathological conditions. Measurement of sodium content revealed that high salt (HS) is a characteristic feature not only in healthy skin, but also in inflamed tissues, lymphoid organs and tumour microenvironment. We have previously shown that HS triggers the production of IL-8 by human neutrophils. Here, we analyzed the transcriptomic profile of neutrophils cultured in HS-supplemented medium (50mM NaCl) for 4hs. We found that HS induced a much more dramatic change in gene expression compared with the conventional agonist LPS (50 nM): (2975 vs 64 genes upregulated and 2468 vs 132 downregulated genes for HS vs LPS). Among upregulated genes, we found 8 chemokines (CXCL6, CXCL3, CCL20, PFAV1, CXCL2, CXCL8, CCL28, CXCL1) to be top enriched. A chemokine array confirmed this observation at protein level, and consistent whit this, we found that the supernatant of HS-stimulated neutrophils harvested at 18h induced a high chemotactic response by freshly isolated neutrophils in a transwell assay: % migration of 60 ± 12 vs 5 ± 4, for supernatants from HS vs untreated neutrophils, $p<0.01$, $n=5$). We also found that mithocondrial gene expression was markedly downregulated in HS-treated neutrophils. Consistent with this we found that HS reduced mithocondrial mass analyzed by mitotraker-green/flow cytometry (% reduction 35 ± 15% compared with untreated neutrophils, $n=7$) together with an increased production of mithocondrial oxidant species analyzed by MitoSoX/flow cytometry: % of positive cells 25 ± 8 vs 5 ± 3, for HS- and untreated neutrophils, respectively ($n=6$). Pharmacological blockade of p38MAPK significantly ($p<0.01$) suppressed the production of mithocondrial oxidants induced by HS (% inhibition > 45, $n=5$). Our results suggest that HS profoundly modulates neutrophil transcriptome inducing dramatic changes in neutrophil behavior.

369. 400. **EVALUATION OF NEUTROPHIL PHENOTYPE IN HEAD AND NECK SQUAMOUS CELL CARCINOMA BASED ON**

HUMAN PAPILLOMAVIRUS INFECTION STATUS AND ITS IMPACT ON TUMOR PROGRESSION

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Background: Head and neck squamous cell carcinoma (HNSCC) has high incidence and mortality rates. Infection with human papillomavirus (HPV) 16 is a risk factor for the development of HNSCC and influences the prognosis. Tumor-associated neutrophils (TANs) are abundant in the tumor microenvironment of HNSCC. HNSCC cases that are HPV- negative, which generally have a poor prognosis, show a higher infiltration of TANs compared to HPV-positive cases. Nevertheless, it remains unclear whether HPV status modulates the phenotype of these TANs and whether this could impact tumor behavior. **Objectives:** To evaluate the effect of HPV-negative and -positive HNSCC cells on CD66b expression in neutrophils, as well as the infiltration of CD66b+ TANs in HPV-negative and - positive HNSCC, and their potential relationship with tumor progression. **Methods:** In vitro, neutrophils were stimulated with the supernatant from HPV-negative and -positive HNSCC cells. Subsequently, CD66b expression in neutrophils was assessed by immunofluorescence, and PD-L1 expression was evaluated by flow cytometry. Automated immunohistochemistry was performed on 24 HNSCC tissue samples to assess the expression of p16, CD66b, and Ki67. Statistical analyses included Student's t-test, Mann- Whitney U test, and one-way ANOVA. **Results:** HPV-negative HNSCC cells induce the overexpression of the activation marker CD66b in neutrophils, along with increased expression of the protumoral molecule PD-L1. Advanced-stage HNSCC tumors (TNM III-IV) exhibit a higher infiltration of CD66b+ TANs compared to early-stage cases (TNM I-II). Overall, HPV-negative HNSCC tends to show a greater infiltration of CD66b+ TANs and increased tumor proliferation. **Conclusion:** Compared to HPV-positive HNSCC, HPV- negative HNSCC cells induce increased activation of TANs, which, by promoting a state of chronic inflammation, may contribute to tumor progression and impact the clinical outcomes of patients. These findings suggest the potential for developing biomarkers and therapeutic strategies to modulate the infiltration and activation of TANs in HNSCC.

370.411. EFFECT OF LIPOIC ACID IN THE CONTROL OF REACTIVE OXYGEN SPECIES AND NET FORMATION

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Background: Neutrophils are one of the most important cells of innate immunity, primarily to defend the host against pathogens. They achieve their functions through different mechanisms such as phagocytosis, degranulation, and release neutrophil extracellular traps (NETs). During NET formation reactive oxygen species (ROS) are produced to eliminate pathogens by inducing oxidative burst. However, they can also cause host tissue damage and promote the pathogenesis of various autoimmune and inflammatory diseases. Neutrophils present an antioxidant system to face and neutralize excessive ROS self-damage. The lipoic acid (LA) is an endogenous antioxidant synthesized inside cells that can recycle other antioxidants that can help maintain the redox balance. **Objective:** We investigated the effect of LA in the decrease the release of NETs and ROS production, in neutrophil stimulated with bacteria. **Methods**: Neutrophils were isolated from peripheral blood, pre-loaded with different concentration of LA (0.05 mM, 0.5 mM and 5mM), for 60 minutes. Later NETs were induced by incubation neutrophils with *E. coli* and LPS from *E. coli* for 180 minutes. The NET formation was assed by fluorometry using Sytox Green, and fluorescent microscopy with DAPI, in the same way the production of ROS was determined by means of DCFDA that is oxidized by ROS to DCF a fluorescent molecule. **Results:** LA induce a suppressive effect of NET and decreased ROS production in neutrophils that were stimulated with E. coli and LPS, and additionally we know that the doses used do not present toxicity as they do not interfere with viability and metabolism, data analysis was conducted used One-way ANOVA and in certain cases, data normalization (inverse function). statistical significance was determined based on the following *p*-values: ≤0.03 (*), ≤0.002 (**), ≤0.001 (***), and ≤0.0001 (****). **Conclusion:** The results suggest that LA is an effective antioxidant to regulate the oxidative stress and NET

release.

371. 430. **BETA DEFENSINS IN VULVOVAGINAL CANDIDIASIS: PATHOGENESIS, HOST REGULATION AND ANTIFUNGAL POTENTIAL**

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Vulvovaginal candidiasis (VVC) is a prevalent inflammatory condition affecting up to 75% of women of reproductive age, with 9% experiencing recurrent episodes. *Candida albicans* (*Ca*) is the primary causative agent, making VVC the second most common form of vaginitis. Beta defensins (BDs) are antimicrobial peptides (AMPs) known for their microbicidal, chemotactic, and immunoregulatory properties. We aimed to investigate how different host microenvironments regulate the expression of BD1 (constitutive) and BD3 (inducible) during VVC and the *in vitro* ability of recombinant hBD1 and hBD3 to kill *Ca*. Female C57BL/6 (WT), NLRP3$^{(-/-)}$, and IL- 17RA$^{(-/-)}$ mice in estrus (estradiol-treated) were intravaginally inoculated with *Ca-SC5314* on Day 0(D0). Uninfected (TRAT) animals served as controls. Cervicovaginal lavages (CVL) were collected to assess fungal burden (CFU), PMN recruitment, and mBDs expression by Immunofluorescence (IF). Results showed no differences in CFU counts between strains on D2 and D4 post- infection (pi), with a reduction in IL-17RA$^{(-/-)}$ and NLRP3$^{(-/-)}$mice compared to WT on D8 pi. mBD1 and mBD3 expression significantly increased in WT mice at D2 pi compared to TRAT($p<0.0001$ for both BDs) and declined on D4 and D8. Early mBD1 expression(D2) in defective mouse strains showed significant reduction compared to WT (IL-17RA$^{(-/-)}p<0.0001$; NLRP3$^{(-/-)}p<0.0001$), while mBD3 decrease exhibited varying dependence on the animal's genetic background(IL-17RA$^{(-/-)}p<0.0001$;NLRP3$^{(-/-)}p<0.05$). These results demonstrate the relevance of IL-17 and IL-1β on BDs expression during VVC. We also assessed the candidicidal activity of both recombinant hBD1 and hBD3 *in vitro*. The *Ca-SC5314* strain treated with hBD3 for 24 hours showed a 98% reduction in CFU compared to the untreated strain($p<0.0001$), with efficacy comparable to fluconazole($p<0.0001$) a classic antifungal drug used for the treatment of VVC patients. A thorough understanding of BDs is essential for grasping the pathogenesis and advancing the treatment of VVC, highlighting their potential as a therapeutic alternative amid rising azole resistance.

372. 482. **ROLE OF ANTIBIOTIC-INDUCED DYSBIOSIS AND CLOSTRIDIOIDES DIFFICILE ON GUT-LUNG CROSSTALK: IMPACT ON SLAMF1 EXPRESSION AND MACROPHAGE FUNCTIONS AGAINST MYCOBACTERIUM TUBERCULOSIS**

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The gut-lung axis involves cross-regulation of microbiomes and/or their metabolites and immunomodulatory signals between the gut and lung. This study addressed the axis in the context of Tuberculosis (TB), the leading cause of death from a single infectious agent, and *C. difficile* infection (CDI), the major cause of antibiotics-associated diarrhoea. We aimed to evaluate the impact of antibiotic-induced dysbiosis and CDI on lung and gut tissues, as well as on macrophage functions against *M. tuberculosis*. A murine model was used to evaluate histopathology and expression of the microbial sensor SLAMF1. Human monocyte-derived macrophages (MΦ) were trained with heat-inactivated *C. difficile* (CDH) for 1, 2, 5 or 7d and then exposed to gamma-ray inactivated *M. tuberculosis* (WCMtb) for 1 or 24h. The endocytic capacity and SLAMF1 levels were assessed by flow cytometry and fluorescent microscopy. In mice, dysbiosis impaired gut integrity and CDI exacerbated histopathological damage. Despite partial recovery from infection, dysbiosis-induced histological changes persisted. Moreover, dysbiosis caused structural lung damage that could not be recovered on *C. difficile*-infected mice. While initial dysbiosis did not affect SLAMF1 expression, SLAMF1 levels increased in the colon on day five post-infection or post-dysbiosis. In humans, CDH endocytosis remained unchanged during training. However, MΦ that internalized CDH lost the ability to uptake WCMtb. Consequently, MΦ that exclusively endocytosed WCMtb increased upon

training. SLAMF1 expression peaked on day 5 of the training and was induced by 1h of WCMtb exposure. In the presence of CDH, SLAMF1 decreased and even longer stimulation with WCMtb failed to induce its expression. CDH training also disrupted SLAMF1 co-localization with WCMtb. In conclusion, our results suggest that *C. difficile* interferes with protective immune mechanisms, affecting the lung and the MΦ responses against *M. tuberculosis* and highlight the role of the gut-lung axis in the development of CDI and TB.

373. 493. **IL-10 MODULATES SUSCEPTIBILITY TO SHIGA TOXIN TYPE 2 AND LEUKOCYTE DYNAMICS IN IL-10-DEFICIENT MICE**

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Shiga toxin (Stx)-producing *Escherichia coli* infections may cause hemolytic anemia, thrombocytopenia, and renal failure, leading to Hemolytic Uremic Syndrome (HUS). Previously, we demonstrated that IL-10 deficient (IL-$10^{-/-}$) mice are protected against Stx. Considering this background, we aimed to further evaluate the role of IL-10 in this strain. IL-$10^{-/-}$ mice were inoculated with either an IL-10-producing plasmid (PL) or saline. After 48 hours, Stx (1 ng/mouse) or saline were administered (experimental groups: Control, PL, Stx, and Stx+PL). Blood and kidneys were extracted 72 hours later. Higher plasma urea levels were observed in the plasmid group (mg/dL) [Median (IQR) = Basal: 53.80(51.97- 61.13); PL: 75.60(40.00-80.13) Stx: 118.10(83.80-133.30); Stx+PL: 220.90(169.10- 292.50)*; n=6; *p<0.05], indicating greater kidney damage and that IL-10 increased the susceptibility to Stx. To extend our study, we analyzed monocytes and PMN in the kidneys by flow cytometry, since alterations in leukocytes are associated with HUS. Following Stx administration, the plasmid group showed an increased percentage of classical monocytes (inflammatory, Ly6C+CD43-) [median(IQR) = Basal: 38.10(35.40-42.10); PL: 46.00(62.40- 57.10); Stx: 56.15(55.00-63.00); Stx+PL: 87.40(79.50-93.50)*; n=6; *p<0.05] and a reduced percentage of non-classical monocytes (anti-inflammatory, Ly6C-CD43+) [median(IQR) = Basal: 27.90(24.30-30.10); PL: 20.90 (17.30-26.10); Stx: 22.90 (19.38-25.83); Stx+PL: 10.18 (6.79-11.68)*; n=6; *p<0.05], suggesting more inflammation with IL-10. Additionally, activation markers CD11b and CD43 were assessed in PMN. CD11b was upregulated [median(IQR) = Basal: 21,660(17,500-22,408); PL: 22,530(21,580-23,17); Stx: 23,100(20,750-24,720); Stx+PL: 27,780(23,500-29,290)*; n=6; *p<0.05] and CD43 was downregulated [median(IQR) = Basal: 5719(5040-5927); PL: 5705(4830-6031); Stx: 4036(3597-5003); Stx+PL: 3366(3294-3519)*; n=6; *p<0.05], indicating a higher state of activation of PMN in the presence of IL-10. These results suggest IL-10 could participates in monocyte differentiation/recruitment and PMN activation. Our results demonstrate that IL-10 reversed the protection observed in IL-10-/- mouse. Furthermore, the alterations observed in monocytes and PMN contributes to a deeper understanding of the immune mechanisms involved in Stx-mediated pathogenesis.

374. 508. **INTESTINAL DYSBIOSIS ORCHESTRATED BY ESCHERICHIA/SHIGELLA, PREVOTELLA AND DEPLETION OF SHORT-CHAIN FATTY ACID-PRODUCING BACTERIA ? THE TRIAD ASSOCIATED WITH EXHAUSTED NK CELL PHENOTYPE IN PATIENTS WITH CERVICAL CANCER IN WESTERN MEXICO: A PIONEERING STUDY AT A GLOBAL LEVEL**

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Background: The intestinal microbiota (IM) plays

an important role in the immune regulation of NK cells, which are key mediators of the anti-tumor response. To date, the interaction between the intestinal microbiota and the exhausted phenotype of NK cells in cervical cancer (CC) is unknown. **Objective**: To characterize the profile of the IM in patients with CC and healthy controls, and its association with peripheral and intratumoral NK cells with exhausted phenotype. **Material and methods**: Cross- sectional study that included 20 patients with a histopathological diagnosis of CC recruited at the Instituto Jalisciense de Cancerología, and 15 healthy women. Stool samples were taken to characterize the IM by NGS sequencing of 16S-rRNA gene. Peripheral blood and tumor tissue were collected to analyze the exhausted immunophenotype of peripheral or intratumoral NK cells by multicolor flow cytometry. **Results**: The intestinal microbiota of patients with CC revealed a significant expansion of LPS-carrying pathobiont bacteria: *Prevotella*, *Escherichia*/*Shigella* and *Desulfovibrio*, plus a decrease in beneficial bacteria producing short-chain fatty acids: *Agathobacter*, *Lachnospira* and *Alistipes*. Predicted bacterial metabolic pathways showed an LPS-induced proinflammatory profile in CC. The exhausted immunophenotype analysis at the peripheral level showed a predominance of $CD56^{bright}$ NK cells only positive for PD-1, TIM-3 or LAG- 3. Considering that the exhausted phenotype is defined as the persistent expression of multiple inhibitory receptors, the co-expression analysis was performed showing populations of $CD56^{bright}PD\text{-}1^{+}LAG\text{-}3^{+}$, $CD56^{bright}PD\text{-}1^{+}TIGIT^{+}$ and $CD56^{bright}PD\text{-}1^{+}TIM\text{-}3^{+}$ NK cells. This phenotype was more pronounced in tumor-infiltrating cells, where populations of $NKCD56^{bright}PD\text{-}1^{+}BTLA^{+}$ and $CD56^{bright}PD\text{-}1^{+}NKG2A^{+}$ cells were also found. **Conclusions**: Intestinal dysbiosis in CC with a predominance of LPS-carrying bacteria could facilitate mechanisms involved in cervical carcinogenesis such as inflammation, perpetuation of HPV infection and NK cell exhaustion. Functional studies are needed to demonstrate these important findings

375. 529. PROINFLAMMATORY MESSENGERS: EFFECTS OF PLACENTAL EXTRACELLULAR VESICLES EXPOSED TO HYPERGLYCEMIA AND PORPHYROMONAS GINGIVALIS-LIPOPOLYSACCHARIDE ON GINGIVAL FIBROBLASTS

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Background. Gestational diabetes mellitus (GDM) is linked to periodontitis, partially due to the placental translocation of periodontal bacteria and their by-products, such as *Porphyromonas gingivalis* lipopolysaccharide (*Pg*-LPS). Our previous research has explored how hyperglycemia and *Pg*-LPS activate NF-κB and NLRP3 inflammasome pathways in GDM placental explants. Furthermore, these stimuli may alter the release profile and inflammatory impact of placental extracellular vesicles (pEVs), potentially influencing periodontal tissues through a bidirectional cross-talk. **Objective.** To analyze the uptake and proinflammatory effect on gingival fibroblasts of pEVs from GDM-placental explants subjected to hyperglycemia and *Pg*-LPS. **Method.** Chorionic villi explants from five GDM pregnancies were stimulated with hyperglycemia and *Pg*-LPS. pEVs were isolated from the conditioned medium by ultracentrifugation and characterized by western blot for surface markers, transmission electron microscopy for morphology, and nanoparticle tracking analysis for size distribution. Gingival fibroblasts from healthy donors were then exposed to fluorescently labeled pEVs (n=3). The uptake of pEVs was assessed using confocal microscopy, and the expression of inflammatory cytokines (IL-1β, IL-6, IL-8, MCP-1, TNF-α) was measured by RT-qPCR and multiplex immunoassay. Statistical significance was determined using an unpaired T-test. **Results.** pEVs exhibited surface markers CD63, CD9, CD81, Syntenin-1, and ALIX, a round cup-shaped morphology, and a size range of 30-400 nm with a mode of 151 nm. Gingival fibroblasts co-cultured with fluorescent pEVs showed a significantly higher mean fluorescence intensity compared to controls ($p=0.008$), and were localized within both the nucleus and cytoplasm of

fibroblasts. Additionally, pEVs increased mRNA levels of IL-6 (*p=0.0321*) and IL-1β (*p=0.05*) and elevated protein levels of IL-6 (*p=0.0041*), IL-8 (*p=0.0186*), TNF-α (*p=0.0052*), and MCP-1 (*p=0.0922*) in the fibroblast-conditioned medium. **Conclusion.** pEVs from GDM placental explants are uptaken up by gingival fibroblasts and induce their proinflammatory response. These findings suggest a potential mechanism linking periodontitis and GDM through the effects of pEVs.

376. 531. PLATELETS MODULATE THE EFFECTS OF NEUTROPHIL EXTRACELLULAR VESICLES RELEASED IN RESPONSE TO SHIGA TOXIN ON RENAL EPITHELIAL CELLS

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Infections by Shiga toxin-producing *Escherichia coli* (STEC) can cause from self-limiting diarrhea to Hemolytic Uremic Syndrome (HUS), a condition characterized by non-immune hemolytic anemia, thrombocytopenia, and acute renal failure. STEC colonizes the intestine, releasing Shiga toxin (Stx), which translocates into the circulation and transits associated to blood cells and their extracellular vesicles (EVs) to its target organs. Stx cytotoxicity on glomerular endothelial cells and tubular epithelial cells (TEC), promotes platelet adhesion, and thrombi formation. Stx also stimulates pro-inflammatory cytokines' release that fosters leukocyte recruitment, platelet aggregation, and fibrin deposition, causing vessels' occlusion and consequently hemolytic anemia. We previously determined that neutrophils release EVs either spontaneously (EVs-C) or in response to Stx2 (EVs-Stx), and that EVs-Stx contain Stx and exert cytotoxicity on human proximal TEC (HK-2). Here we aimed to evaluate whether platelets could modulate the effects of EVs-Stx on HK-2. To this end, we purified platelets and neutrophils from peripheral blood of healthy donors and isolated EVs-C and EVs-Stx released from 10^7 neutrophils after stimulation with Stx2 (0.1µg/ml). First, we determined by live-cell microscopy that EVs-Stx are incorporated by HK-2 cells and confirmed their capacity to trigger cell death. Then, we seeded the EVs onto HK-2 cells with or without autologous platelets and after 16h we determined IL-8 concentrations and LDH activity in supernatants. EVs-Stx did not modulate HK-2 IL-8 secretion but triggered LDH release at similar levels as Stx2 alone. Surprisingly, platelets promoted the secretion of high IL-8 levels by HK-2 in the presence of EVs-Stx (n=5, p<0.01) but not when incubated with or without HK-2 cells alone. Additionally, platelets tended to reduce but did not significantly modulate the HK-2 lytic death induced by EVs-Stx (n=4). The ability of platelets to modulate the effects of EV-Stx on renal epithelial cells might contribute to thrombotic microangiopathy in HUS.

377. 548. PROSTAGLANDIN E2 DRIVES A UNIQUE PRO-RESOLVING PROFILE IN HUMAN MACROPHAGES

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Prostaglandin E2 (PGE2), a well-known mediator with pro- and anti-inflammatory properties, is essential for initiating inflammation resolution. Macrophages contribute to this process through the removal of apoptotic cells (efferocytosis) and cytokine secretion. However, the interplay between PGE2 and macrophage resolving features has been poorly studied in humans. We hypothesized that PGE2 reprograms macrophages into a pro-resolving profile. To assess this, human monocytes from buffy coats were differentiated into macrophages over 7 days with M-CSF. On day 5, macrophages were treated with PGE2 (1 µM) until day 7, or left untreated as controls. Efferocytosis was measured by flow cytometry using CFSE-stained apoptotic Jurkat cells, and transcriptional changes were analyzed by RNA-seq. Cytokines were assessed by ELISA. Our results showed that PGE2 enhanced efferocytosis, induced spontaneous secretion of TGF-β and VEGF-A, upregulated surface markers CD14, CD16, and HLA-DR and prevented LPS-induced increases of TNF and IL-6 production. PGE2-induced enhancement of efferocytosis persisted even in the presence of inflammatory stimuli (LPS, zymosan, and resiquimod). A 6-hour

pulse of PGE2 on day 5, followed by 42 hours without treatment, replicated the efferocytosis increase seen with continuous 48-hour PGE2 exposure, indicating that PGE2 induces genetic reprogramming in macrophages. Transcriptome analysis identified 169 differentially expressed genes, with thrombospondin-1 being the most upregulated (7.5-fold, $p<0.01$). However, neither conditioned media from PGE2-treated macrophages nor exogenous thrombospondin-1-enhanced efferocytosis. On the other hand, GSEA revealed enrichment in TGF-β signaling transcripts, and, accordingly, TGF-β receptor blockade with SB431542 abolished the PGE2-mediated increase in efferocytosis. Interestingly, the addition of exogenous TGF-β did not replicate the PGE2 effect, suggesting that PGE2-induced reprogramming requires an additional mediator, yet unidentified, beyond TGF-β. In summary, PGE2 induces a stable and unique pro-resolving profile in human macrophages that resists inflammatory stimuli, through a complex mechanism partially mediated by TGF-β.

378. 549. **THE CRYSTAL STRUCTURE OF CALCIUM PYROPHOSPHATE DIHYDRATES DETERMINES THE TYPE OF INFLAMMATORY RESPONSE IN NEUTROPHILS**

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Calcium pyrophosphate (CPP) deposition disease results from an immune response to the pathological presence of CPP crystals in the joints, leading CPP-crystal inflammatory arthritis. There are four types of CPP crystals: triclinic CPP (t-CPP), monoclinic CPP (m- CPP), amorphous CPP (a-CPP), and monoclinic CPP beta (mß-CPP) crystals. The monoclinic and triclinic phases are the two types of CPP crystals identified in synovial fluids at the time of acute arthritis, which are responsible for recurrent inflammatory flares due to their ability to induce production of a wide array of pro-inflammatory mediators. Additionally, the existence of precursor phases such as mß-CPP and a-CPP have been also suggested. In this study, we aimed to evaluate the potential of the four types of CPP crystal to induce inflammatory responses on neutrophils. To achieve this, each phase of crystal was synthesized and subsequently used to stimulate purified human neutrophils isolated from healthy subjects. We assessed reactive oxygen species (ROS) production and NET formation in basal neutrophils (basal), neutrophils stimulated with each type of CPP crystal, and neutrophils stimulated with PMA. P value is the result of one-way ANOVA (Kruskal-Wallis test) followed by Dunn's post-hoc test. In vitro studies demonstrated that m-CPP and t-CPP induced extracellular DNA release and NET formation ($p\leq0.01$) compared to neutrophils stimulated with mß-CPP and a-CPP ($p>0.2$). Furthermore, m-CPP was the only crystal that significantly enhanced ROS production ($p\leq0.05$). Notably, these differences in inflammatory responses were associated with crystal phagocytosis, as we have shown that neutrophils phagocytose m-CPP and t-CPP crystals ($p\leq0.001$). In conclusion, this study illustrate that neutrophils exhibit distinct inflammatory characteristics depending on the phase of the CPP crystal. The capacity to recognize CCP phases in synovial fluid will enhance patient stratification and may lead to new therapeutic options.

379. 551. **PLASMA EXTRACELLULAR VESICLES FROM LONG COVID PATIENTS PROMOTE NEUTROPHIL AGGREGATION AND CELL DEATH**

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Post-acute COVID-19 condition, or Long COVID, is characterized by persistence or new onset of symptoms after initial SARS-CoV-2 infection. Among symptoms, thrombotic anomalies stand out. Pathophysiological mechanisms involved in this predisposition are unclear. Neutrophils are the most abundant leukocytes in bloodstream and their intravascular activation may promote thrombo-inflammation. Extracellular vesicles (EVs) are nanoparticles produced by virtually all cell types that carry biologically active molecules. Our group demonstrated that COVID-19 survivors carry high levels of EVs expressing tissue factor (TF) in plasma. In addition to promoting coagulation, TF can activate protease activated receptor type 2 (PAR-2), a receptor associated with neutrophil activation. Therefore, we aim to explore the contribution of EVs in the process of neutrophil activation. Neutrophil were purified from healthy donors by density gradient separation and incubated with plasma EVs from healthy controls (EV-CT) or long COVID patients (EV-LC) for 30 to 120 minutes, which were purified by size exclusion chromatography. EV-LC promoted significant neutrophil aggregation at all time points ($p<0.05$) with a peak of aggregation index at 60 min ($p<0.05$) with a significantly greater diameter at 120 min compared to unstimulated cells ($p=0.0571$). EV-LC and EV-CT did not induce NET formation after 90min, despite neutrophil response to lipopolysaccharide. We validated surface expression of TF on EV-LC and observed direct contact of EVs with neutrophil membrane, suggesting fusion. TF accumulation is known to promote cell death, and indeed we observed that EV-LC induced neutrophil cell death at 90min detected by significant LDH release. Hence, we demonstrate that EV-LC promote neutrophil aggregation and cell death, which may contribute to vascular occlusion and activation of thromboinflammation during long COVID. We aim to further characterize EVs, interaction with neutrophils and the role of TF/PAR-2 and leukotrienes in this phenomenon. Our data help shed light into the potential identification of therapeutical targets.

380. 553. **SARS-COV-2 INDUCES MONOCYTES TO RELEASE DNA EXTRACELLULAR TRAPS (DETS)**

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It has been shown that SARS-CoV-2 induces the release of Neutrophil Extracellular Traps (NETs), which are harmful to lung epithelial cells, were evidenced in lung biopsies, and correlated with COVID-19 severity. Similarly to NETs, monocytes release DNA Extracellular Traps (DETs) in response to various stimuli. Here, we evaluate whether SARS-CoV-2 and its spike protein induce the release of DETs in monocytes, determining the mechanisms involved in this process and investigating the possible pathogenic role of these DETs to cells. Our results confirm that human peripheral blood monocytes stimulated by beta- propriolactone-inactivated SARS-CoV-2 and its spike protein release DETs, measured by Picogreen assay. These DETs presented the characteristic morphology observed by scanning electron microscopy (SEM) and immunofluorescence analysis with specific probes and antibodies showed the presence of DNA associated with elastase, myeloperoxidase (MPO), and citrullinated histone H3 (H3Cit) in their composition. SARS-CoV-2 induces the production of reactive oxygen species (ROS) in monocytes, the inhibition of which decreases DET extrusion. We used specific inhibitors to demonstrate that DET extrusion depends on MPO, peptidyl arginine deiminase (PAD), calcium, elastase, gasdermin-D, and NLRP3 inflammasome. Inhibition of TLR2, TLR4, and ACE2 receptors decreases DET release by monocytes. DETs were toxic to lung epithelial (A549 and Calu-3) and endothelial (hBMEC) cell lines as measured by resazurin reduction. DETs pretreated with anti-citrullinated histone antibody and elastase, as well as MPO inhibitors, decreased the toxicity to A549 cells, demonstrating the toxicity of these DET-associated molecules. The results indicate that SARS-CoV-2 induces the release of DETs in monocytes, which may participate in the pathophysiology of COVID-19.

381. 593 **ROLE OF IRE1 RNASE ACTIVITY IN THE DEGRADATION OF MIRNAS IN CONVENTIONAL TYPE 1 DENDRITIC CELLS**

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Background: The Unfolded Protein Response (UPR) is a crucial cellular stress pathway that maintains endoplasmic reticulum (ER) proteostasis. Beyond its canonical role, the IRE1-XBP1 axis of UPR regulates the physiology of conventional type 1 dendritic cells (cDC1). IRE1 degrades RNAs via Regulated IRE1-dependent decay (RIDD). While RIDD importance for cDC1 function is known, its impact on microRNA (miRNA) levels, critical for development, survival, and inflammation, remains unclear. This study investigates whether IRE1, through RIDD, regulates miRNA levels affecting cDC1 function. **Objective:** To determine how RIDD influences miRNA levels related to immune functions (miR-155, pro-inflammatory) and survival (miR-125a, pro-apoptotic) in cDC1. **Methods:** We use an *in vitro* model of cDC1, including conditional knockout mice: CD11c-Cre XBP1 fl/fl (lacking XBP1s, with active RIDD), CD11c-Cre IRE1 fl/fl (lacking IRE1's RNase domain, inhibiting RIDD), and a novel mouse model with a point mutation S729A in the IRE1 RNase domain (selectively inhibiting RIDD). miRNA levels were measured by qPCR, and Flow Cytometry assessed cell viability. **Results:** XBP1-deficient cDC1 showed reduced miR-155 levels compared to wild-type cells, correlating with increased anti-inflammatory mRNAs and reduced IL-12 and TNF- alpha production. Enhanced survival was observed in XBP1-deficient cDC1 following chronic activation and apoptosis induction, which was associated with decreased miR-125a levels. No significant differences were noted between cDC1 cKO IRE1trunc and wild-type cells. Interestingly, XBP1-deficient cDC1 with IRE1 A/A mutation exhibited higher miRNA levels than XBP1 cKO cDC1. **Conclusion:** RIDD activation in cDC1 correlates with reduced miR-155 and miR-125a levels. Lower miR-155 levels increase anti-inflammatory mRNAs, potentially impacting pro- inflammatory responses. Increased cDC1 XBP1 cKO viability may relate to reduced miR-125a. Elevated miR-155 and miR-125a in cDC1 with the IRE1 A/A mutation suggest that RIDD degrades these miRNAs. This study offers new insights into miRNA regulation in cDC1 via RIDD, proposing a novel regulatory axis for therapeutic exploration.

382. 604. EVALUATION OF GENE EXPRESSION PROFILE AND PRO-INFLAMMATORY MOLECULES PRODUCTION IN MICROGLIAL CELLS AFTER TLR2 STIMULATION

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Background: Toll-like receptors (TLRs) ligands activate microglia, triggering inflammatory reactions in the CNS. Mouse experimental models of brain abscesses revealed a complex role for TLRs in disease pathogenesis. TLR2 participates in the innate immune response during brain abscess formation induced by Staphylococcus aureus, influencing the adaptive immune response. Identifying potentially neurotoxic molecules released by TLR2 stimulation may provide insights into neuropathological mechanisms involved in these diseases. Additionally, evaluating the transcriptome of activated microglial cells may reveal complex pathways modulated by TLR2. **Objectives:** Here, we aimed to evaluate gene expression profiles and perform pathway analysis in murine microglial cells following TLR2 stimulation**. Methods:** RNA-seq public datasets from mouse microglial cells and/or macrophages stimulated with TLR2 ligands were obtained from the NCBI Gene Expression Omnibus (GEO) repository and analyzed using the integrated Differential Expression and Pathway (iDEP) analysis tool. For validation experiments, murine BV2 microglial cells were cultured with or without phosphatidyl-inositol-3 kinase (PI3K) inhibitors and then stimulated with Pam3CSK4 or LPS (control) to determine TNFalpha and nitric oxide (NO) production. **Results:** Preliminary exploration of the RNA-seq data showed enhanced pro- inflammatory gene expression in microglial cells stimulated with TLR2 agonists, compared to the control groups. We found enrichment in differentially expressed genes (DEGs) in TNFalpha, NOD-like receptor and NF-kappa B signaling pathways ($p < 0.01$) (KEGG). Visualization of fold-changes of all genes in KEGG pathways suggests that TLR2 induces up-regulation of PI3K, pro-inflammatory cytokines, and molecules associated with the NF-kappa B signaling pathway and Parkinson's Disease ($p < 0.001$). Equivalent results were obtained in transcriptomic analysis and in vitro experiments ($p < 0.001$). **Conclusions:** Transcriptomic analysis of microglial cells suggests that TLR2 stimulation modulates several pro-inflammatory gene expression and cell responses that may be involved in neuroinflammation.

383. 605. **CHARACTERIZATION OF THE EXPRESSION AND FUNCTION OF THE IL-9 RECEPTOR IN MURINE BASOPHILS**

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Infection with *Nippostrongylus* brasiliensis in mice shows an increase in the number of basophils, which is significantly lower in IL-9 deficient mice. In the same phenomenon, the expression of the IL-9 receptor (IL-9R) in basophils was identified; however, in a model of sterile inflammation, the expression of IL-9R is not induced, leaving questions about the underlying mechanism of IL-9R expression in the presence of the helminth and the function of the cytokine in basophil physiology. Firstly, the induction of basophils expressing IL-9R in lungs infected by *N. brasiliensis* over time was characterized using flow cytometry. We found the highest number and frequency of basophils on day 5 post-infection significantly. On the other hand, with the sterile inflammation model, it was confirmed at the protein level by flow cytometry that IL-9R is not expressed in basophil membranes in the absence of the helminth. When evaluating the survival and proliferation of pulmonary basophils in the presence or absence of IL-9, we found that this cytokine, along with IL-3 and TSLP, significantly increases the proliferation of basophils *ex vivo* and there is a tendency to increase the number of living basophils in culture. Finally, we evaluated *in vitro* differentiated basophils deficient in the MYD88 protein, essential for signaling via Toll-like receptors that could recognize helminth- associated molecular patterns. We found that the receptor induction pathway is affected; however, this protein does not mediate the mechanism of IL-9R induction. This work is the first to identify the presence of IL-9R at the protein level in the membrane of basophils, as well as to describe its role as a growth factor in these cells.

384. 607. **STUDY OF THE TGF-BETA-INDUCED SIGNALING IN NK CELLS: CHARACTERIZATION OF THE TIF1-GAMMA-DEPENDENT PATHWAY**

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TRIM33, or TIF1-gamma, is part of the TGF-Beta pathway. It is involved in DNA repair, anaphase, apoptosis, ubiquitination of Smad4/Beta-catenin and due to its structure, acts as a chromatin reader. In immune cell populations, the role of TRIM33 remains to be fully explored. This project, therefore, investigates the function of this protein on TGF-beta-induced NK phenotypes. The basal expression of TRIM33 in T and NK cells from mouse spleen was analyzed using flow cytometry and qPCR. The alpha and beta isoforms were identified in NK cells using densitometry. In order to assess the role of TRIM33 in these cells, we used tamoxifen-induced conditional TRIM33 KO mice. In parallel, we knocked out TIF1- gamma from NK cells with CRISPR/Cas9. The production of cytotoxic molecules, as well as the eVects on survival and proliferation were evaluated on KO vs WT cells. We found higher expression of TRIM33 in CD8+ cells compared to NK cells. Similar to T cells, both the alpha and beta isoforms were expressed in NK cells. TRIM33 deletion was successfully achieved with the tamoxifen model and with CRISPR/Cas9, although the latter resulted in a low percentage of edited cells (10%-15%). TRIM33 KO cells obtained using both methods showed reduced viability, proliferation, and release of granzymes, perforins, and interferons after stimulation. These results contribute to our understanding of the role of TRIM33 in NK cells, a population were this protein had not been explored. A full characterization of NK cells is of great importance because of their therapeutic potential.

385. 632. **ASSOCIATION BETWEEN MATERNAL OBESITY AND THE PHENOTYPE OF MONOCYTES AND HEMATOPOIETIC PROGENITOR CELLS IN THEIR OFFSPRING AT BIRTH**

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Background: Maternal-obesity is characterized by metabolic-endotoxemia (elevated blood lipopolysaccharides levels) and is associated with immune dysfunc0on of the offspring. Monocytes are immune cells that are derived from hematopoie0c progenitor cells (HPCs) and develop in early embryonic stages. Thus, the study of these cells would help iden0fy the origin of fetal immune programming by maternal-obesity. **Objec0ves:** To evaluate whether maternal-obesity is associated with metabolic endotoxemia, changes in immunophenotype, gene expression, DNA-methyla0on paaerns, and func0on of HPCs and monocytes from their offspring at birth, compared to normal- weight women (NW). **Methods:** Pregnant women with maternal-obesity and NW (n=21, both groups) were recruited (ethical-approval #200920001). At delivery, umbilical-cord blood (UCB) was collected to determine lipopolysaccharide levels (photometry), and HPCs and monocytes were isolated to measure immunophenotypes (flow-cytometry), transcript levels (RT-qPCR), global DNA-methyla0on (EPIC-850K,Illumina®), PPARγ-methyla0on (pyrosequencing), and *invitro l*ipopolysaccharide effect (RT-qPCR, flow-cytometry). Sta0s0cs: group comparisons (Mann-Whitney test), EPIC array (Limma-voom, normalized M-values), significant ($p<0.05$), and FDR-adjustment. **Results:** Compared to NW, the maternal-obesity group had higher lipopolysaccharide levels in their offspring. Also, have a greater number of UCB-HPCs early myeloid lineage, and UCB- monocytes with elevated transcripts levels of IL-6, IL-1β, and MCP1, decreased PPARγ levels, global DNA hypomethyla0on, and a blunted response to lipopolysaccharide *invitro*. Both UCB-monocytes and HPCs, had higher PPARγ gene methyla0on. **Conclusion:** The offspring of women with obesity present metabolic-endotoxemia, and phenotypic, epigene0c, and func0onal changes in their immune cells, which suggest a fetal programming by maternal-obesity. Our data provide a molecular argument for including preconcep0on interven0ons to prevent the effects of maternalobesity on the offspring.

386. 657. TROGOCYTOSIS OF NEUTROPHIL SURFACE RECEPTORS BY CANDIDA ALBICANS

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By trogocytosis, cells acquire membrane fragments from donor cells via a contact-dependent mechanism, allowing the acquisition of functional receptors and thus modulating the function of receptor cells. Trogocytosis has shown to be involved in a variety of processes such as cell-cell communication, antigen presentation, immune regulation, and anti-tumor immunity. The mechanisms underlying trogocytosis are still poorly characterized. Trogocytosis has been mainly analyzed in the context of interacting immune cells. Here, we analyzed whether the human pathogen Candida albicans (CA) could trogocyte cell surface receptors expressed by neutrophils. Human blood samples were obtained from healthy donors and neutrophils were isolated by conventional methods. Neutrophils and CA were cultured in a 1:10 ratio for 1 h at 37°C, in RPMI medium supplemented with autologous serum (5%). Then, the expression of CD11b and CD16 was analyzed by flow cytometry in the gate containing only free CA (non-phagocyted CA). As expected, CA cultured alone expressed neither CD11b nor CD16 (% expression <1%, n=6). By contrast, after culture with neutrophils, CA expressed on its cell surface both, CD11b and CD16: % free CA positive for CD11b and CD16 expression = 65 ± 18 and 25 ± 7 (mean ± SE, n=5, $p<0.001$ vs CA cultured alone). Analysis performed by confocal microscopy revealed a pattern of transference compatible with trogocytosis (not shown). Depletion of serum antibodies directed to CA markedly reduced the efficiency of trogocytosis: % free CA positive for CD11b = 71 ± 15 vs 22 ± 11 (mean ± SE, n=4, $p<0.01$ untreated serum vs serum depleted of anti-CA antibodies). Our results suggest that CA can trogocyte cell-surface molecules expressed by neutrophils by a mechanism depen-

dent on the presence of anti-CA antibodies. Further studies are required to analyze the impact of this mechanism on CA pathogenicity.

387. 693. THE EXTENT OF NEUTROPHIL SERINE PROTEASES ACTIVATION DETERMINES IL-1 BETA SECRETION LEVELS AND NETOSIS

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Neutrophils release extracellular traps (NETs) that trap and kill bacteria and also secrete Interleukin-1 beta (IL-1β), a pro-inflammatory cytokine that shapes immune responses. We previously showed that pro-IL- 1β in human neutrophils is processed into its mature form by both elastase, which exits from azurophil granules to the cytosol, and caspase-1. NETosis and pro-IL-1β processing involve Gasdermin-D pores that allow the serine protease (SP) elastase release from granules into the cytosol, where it can process pro-IL- 1β, but if excessively activated can also lead to its degradation. In previous studies, we found that IL-1β release was high when neutrophils were stimulated with LPS+PMA (2.5 ng/ml)+ATP, but it decreased as PMA concentration rose to 25 ng/ml, a dose that usually triggers NETosis. Thus, we hypothesized that the intensity of the stimuli might inversely determine the extent of NETosis and IL-1β release. Here we investigated the molecular bases of our findings to provide support for the hypothesis proposed. We conducted experiments with neutrophils isolated from healthy donors' peripheral blood stimulated with increasing PMA concentrations (2.5-25 ng/ml). We confirmed by confocal microscopy evaluation that the percentage of NETotic cells was significantly higher at 25 ng/ml ($p<0.05$) while no significant NETosis levels were observed at PMA 2.5 ng/ml. Caspase-1 positive cells were also significantly higher at LPS+ATP+PMA 25 ng/ml ($p<0.05$). We reasoned that increased caspase-1 activation may favor more Gasdermin-D pore formation and elastase release to the cytosol. Supporting this possibility, a higher SP activity was observed at PMA 25 ng/ml ($p<0.0001$). Moreover, a SP inhibitor (SPi) concentration-dependently reduced NETosis, and consistent with the fact SP are required for IL-1β secretion, increasing concentrations of SPi gradually reduced IL-1β release induced by LPS+ATP+PMA 25 ng/ml ($p<0.005$). Altogether, our findings support that a higher intensity of the stimulus increase SP activity while reducing IL-1β secretion but augmenting NETosis.

388. 705. INTRACELLULAR CHOLESTEROL ACCUMULATION AND ITS ASSOCIATION WITH INFLAMMASOME ACTIVATION IN IBD PATIENTS

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Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic inflammation and an aberrant interaction between the immune response and the intestinal microbiota. Recent studies suggest that intracellular cholesterol is related to the immune response in IBD, a possible activator of the NLRP3 inflammasome, a complex inducing the secretion of IL-1β and IL-18. It is still unclear whether cholesterol acts as a DAMP to activate this inflammasome in IBD. The main objective of this study was to evaluate the relationship between cholesterol metabolism and NLRP3 inflammasome activation in patients with IBD. RNA-Seqs from IBD individuals were analyzed to evaluate the expression of genes associated with cholesterol metabolism and inflammasome. Ex-vivo cultures of colonic biopsies from UC, CD patients, and healthy individ-

uals were treated with beta-methyl-cyclodextrin (βMCD) to explore the effects on cholesterol, IL-1β, and IL-18 levels. Statistical analysis was performed with GraphPad Software 10.0, evaluating normal distribution. To compare groups, parametric data was analyzed with t-test or ANOVA and non-parametric data with Mann-Whitney or Kruskal-Wallis tests, considering a significant p-value less than 0.05. Our results from RNA-seq analysis of the 1000 IBD individuals database showed decreased expression of cholesterol efflux genes *ABCA1* and *ABCG1* in inflamed UC patients, and increased intracellular trafficking genes such as *STARD3, NPC1*, and *GRAMD1A* in inflamed UC and CD. Inflammasome components, such as *IL-1β, IL-18*, *CASP1* were also increased in UC and CD. Acute cholesterol depletion with βMCD reduced IL-18 levels in UC biopsies. These results suggest that intracellular cholesterol accumulation may activate the inflammasome, aggravating inflammation in UC. We believe that targeting cholesterol modulation could offer a potential therapeutic strategy for UC patients.

389. 709. **MONOCYTES AND MICROGLIAL CELLS ARE ACTIVATED BY PERIODONTAL EXTRACELLULAR VESICLES AND ACQUIRE A POTENTIALLY NEUROINFLAMMATORY PROFILE**

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Background. Brain-resident and infiltrating myeloid cells, such as microglial cells (MCs) and monocytes are crucial in central nervous system immunity, regulating inflammation towards injury or repair. During pathological neuroinflammation—a common feature of neurodegenerative diseases—MCs adopt an activated or reactive phenotype, characterized by retraction of cytoplasmic extensions and an ameboid morphology with pro-inflammatory functions. Additionally, the breakdown of the blood-brain barrier allows monocytes to infiltrate the brain parenchyma, exacerbating tissue damage. Periodontitis, a chronic inflammatory disease of the gums, has been linked to neurodegenerative disorders. However, the molecular mechanisms underlying this association are not yet fully understood. We hypothesize that periodontal extracellular vesicles from the periodontal bacteria *Porphyromonas gingivalis* (*Pg*) and periodontitis-affected patients (Perio-EVs) activate both monocytes and MCs, promoting an inflammatory profile. **Objective.** To assess whether Perio-EVs can activate and induce an inflammatory response in monocytes and MCs. **Methods.** *Pg*-derived outer membrane vesicles (OMVs), were isolated by ultracentrifugation from *Pg* bacterial cultures supernatant and from gingival crevicular fluid (GCF) samples of periodontitis (Perio) patients or gingivally healthy (GH) subjects. Human THP-1 NF-κB and IRF dual reporter monocytes and HMC3 microglial cells were stimulated with increasing concentrations of *Pg*-OMV*s*, Perio, or GH GCF- EVs. Monocyte activation was assessed by NF-κB and IRF pathway activation, while MCs polarization by morphology, flow cytometry and cytokine secretion. **Results.** *Pg*-OMVs and Perio-EVs increased NF-κB and IRF monocytic activity at all concentrations, whereas GH-EVs only did so at higher concentrations. Notably, the expression of CD11b, a monocyte phenotypic/activation marker, was upregulated accordingly. Similarly, in response to *Pg*-OMVs and Perio- EVs, MCs acquired an active/reactive morphology characterized by a smaller area, reduced perimeter, increased circularity and secretion of pro-inflammatory cytokines (IL-6, TNF-α, and MCP-1). **Conclusion.** Monocytes and MCs are activated by Perio-EVs and acquire an inflammatory profile, supporting a novel mechanism that potentially links periodontitis to neuroinflammation.

MUCOSAL IMMUNOLOGY

390. 028. **MATERNAL OBESITY ASSOCIATES WITH ALTERED HUMORAL IMMUNITY IN COLOSTRUM**

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Negative infant outcomes have been correlated to maternal obesity, a condition with increasing prevalence. The impact of maternal obesity on the humoral response in colostrum remains unclear. We recently identified that the colostrum from mothers with obesity contained a significantly reduced fraction of B lymphocytes ($CD19^+$). Here we performed an observational cross-sectional study, where peripheral blood and colostrum samples from 42 mothers with BMI between 18.5-25 or >30 Kg/m^2 were collected 24-48h *postpartum*. Fourteen B lymphocyte subtypes were investigated using flow cytometry. IgG, IgA, and IgM concentrations were measured using ELISA, and antibody production from colostrum cells were quantified with ELISpot. Maternal obesity correlated with significant regulations of the humoral immune system in both tissue types. In colostrum, isotype-switched memory B lymphocytes ($CD19^+$, $CD27^+$, IgD^-) constituted the most abundant cell subtype overall. The colostrum from mothers with BMI>30 Kg/m^2 contained a significantly larger fraction of isotype-switched memory B lymphocytes (fraction of colostrum $CD19^+$ cells, mean 66.73% vs. 52.02%, p=0.0001), higher *in situ* production of IgG from colostrum cells, and total secretory IgG concentration. Furthermore, unlike mother with BMI between 18.5-25 Kg/m^2, mothers with obesity failed to mount an antibody response against SARS-CoV-2 RBD, in either tissue type, post-Pfizer BioNTech bivalent vaccine administered in the third trimester of pregnancy. This is the first characterization of B lymphocyte subpopulations in colostrum *postpartum*. This work uncovers maternal obesity as a possible modifier of breastmilk immune components. As B lymphocytes and antibodies play a crucial role in regulating neonatal immunity and intestine development, this report urges more research to be performed to evaluate possible outcomes for suckling infants from mothers with obesity.

391. 099. EFFECT OF PROBIOTICS IN THE PROTECTION OF INTESTINAL BARRIER FOLLOWING YERSINIA PSEUDOTUBERCULOSIS INFECTION

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The intestinal barrier is constantly exposed to pathogenic, dietary, and microbiota-derived antigens, which influence tissue homeostasis. Our group has been studying how probiotics, dietary changes, or infection episodes, shape the gut-associated mucosal immune system. We observed that, after infection clearance, *Yersinia pseudotuberculosis* (YP) causes a permanent remodeling of the immune and lymphatic systems of the gastrointestinal tract. This process, named by us “immunological scarring”, is directly related to susceptibility to experimental colitis as it compromises the migration of tolerogenic dendritic cells (DCs) to mesenteric lymph nodes. Here, we tried to reverse the immunological scarring by using the probiotic *Saccharomyces cerevisiae* (SC) UFMG A-905. We hypothesized that the probiotic could recover the mesenteric lymphatic integrity, and the migratory capacity of the CD103+ DCs, crucial for inducing tolerogenic responses in the intestine. Testing on C57BL/6 mice involved two strategies: (1) treatment 4 weeks post-infection, to reverse the immunological scar, and (2) treatment one week before YP infection, continuing until pathogen elimination to prevent its establishment. We examined gut, mesentery, and mesenteric lymph nodes via histopathology and flow cytometry. The results were compared using analysis of variance (ANOVA) followed by the Tukey test. Contrary to our hypothesis, both strat-

egies not only did not protect the animals but also worsened the chronic inflammation caused by the immunological scar, indicated by increased neutrophil and Th1 recruitment to the mesentery and the mesenteric lymph nodes post-infection. The treatments also failed to recover the integrity of the mesenteric lymphatic vessels, since treated mice showed a reduced frequency of CD11b+CD103+ and CD103+ DCs in the lymph nodes. We believe that, in this context, probiotic consumption may increase the bacterial load translocated to other organs due to damage to the intestinal barrier.

392. 106. **EPIGENETIC CONTROL OF CD8+ T CELL TISSUE HOMING AND TISSUE RESIDENT MEMORY T CELL PRECURSORS DIFFERENTIATION BY THE HISTONE METHYLTRANSFERASE SUV39H1**

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Introduction: After infection or vaccination, antigen-specific T cells expand and di:erentiate into central memory (TCM) and e:ectors (TE$) cells. Some of these cells exit lymph nodes (LN) and home to non-lymphoid tissues, where they di:erentiate into tissue-resident memory T cells (TRM). Once established, TRM are among the first to respond during secondary antigen encounters, providing local protection upon reinfection. While it is known that TRM precursors seed tissues during the e:ector phase of immune responses, the precise nature of the TRM precursors and their relationship to circulatory T cells, is still a matter of debate. The di:erentiation of e:ector and memory T cell subtypes is accompanied by profound changes in the epigenetic landscape that maintain gene expression programs. Studies on epigenetic changes during TRM formation revealed both shared and tissue-specific epigenetic signatures, some of which occur early during immune responses, supporting the existence of poised TRM precursors among tissue homing cells. Post-translational modifications of histone N-terminal tails regulate many features of chromatin biology and gene expression and play crucial roles in cell development, including in T cells. Suppression of position e:ect variegation 3-9 1 (SUV39H1) was the first mammalian histone lysine methyltransferase (KMT) identified. Together with its mammalian paralog SUV39H2, SUV39H1 introduces the second and third methyl groups on lysin 9 of histone H3 tails (H3K9me2 and H3K9me3). Through its KMT activity, SUV39H1 plays essential roles in heterochromatin formation and spreading and is one of the most conserved epigenetic silencing systems. Previous work from our laboratory showed that H3K9me3 and SUV39H1 silence Th1- related genes during $CD4^+$ T cell commitment to Th2 cells. More recently, we showed that during e:ector $CD8^+$ T cell di:erentiation, SUV39H1-mediated silencing of stemness genes is necessary to promote the establishment of cell identity, leading to the accumulation TCM cells in lymphoid tissues in the absence of SUV39H1. Finally, we also showed that in tumor models, including CAR T cells, SUV39H1 defect leads to a dual phenotype, increased TCM in lymphoid organs and increased e:ectors in tissues. Here, we aimed to explore the role of SUV39H1 in T cell tissue homing and residence. Methods: We characterized CD8+ T cells residing in di:erent tissues of SUV39H1-KO and conditional SUV39H1-flox-CD4-Cre mice (Cre+) and WT littermates (WT/Cre-) at steady state and upon influenza infection by flow cytometry. To validate tissue homing of cells, we stained cells intravascularly (iv) with anti-CD45 or anti-CD8alpha mouse antibodies for 5' before mice euthanasia. We also explored tissue homing and TRM di:erentiation of SUV39H1-KO or WT TCR transgenic OT-I CD8+ T cells upon adoptive transfer into lymphopenic mice and upon SIINFEKL-expressing influenza virus infection by flow cytometry and CITE-seq. To investigate circulatory replenishment of tissue homing cells, we treated mice with FTY720 to inhibit lymphocyte recirculation 30 days after adoptive transfer. To evaluate the functionality of the SUV39H1 KO TRM cells in lungs after flu infection, mice were infected with influenza A/X-31(X31) and rechallenged 28 days after with influenza A/PR8 (PR8). Finally, to analyse the capacity of lung-homing cells to delay lung tumor metastases we transferred naïve OT-I $CD8^+$ T cells from SUV39H1-KO mice or WT littermates into RAG-KO mice and between 33 and 35 days later, we challenged the mice with an iv. injection of B16 melanoma cells expressing SIINFEKL and luciferase (B16-OVA-Luc). Experiments were repeated at least twice, except for CITE-seq experiment that was done just once. Results are shown as median(lower quartile-higher quartile). Some results are expressed as the normalized value to the mean of the WT control

group of each experiment. Results: To investigate whether SUV39H1 participates to the di:erentiation of TRM cells, we first quantified $CD8^+$ T cells in tissues at steady state in SUV39H1-KO mice and WT littermates. $CD62L^-$ $CD44^+$ $CD8^+$, but not $CD4^+$, T cells accumulated in SUV39H1-deficient mice in non-lymphoid organs as the liver (WT: 28,5%(19-48.8); KO: 57.1%(47.7-72.4). $CD62L^-$ $CD44^+$ $CD8^+$ T cells also accumulated in the liver [Cre+: 44.75%(38-50.88); Cre-: 21.2%(18.93-24.25)] and lung [Cre+: 2.84%(2.74-2.96); Cre-: 1.42%(1.09-1.73)] of conditional SUV39H1-deficient (Cre+) mice.

The iv- fraction of lung $CD8^+$ T cells was increased in Cre+ mice, both in percentage [Cre+: 1.05%(0.81-4.82); Cre-: 0.62%(0.29-1.08)] and numbers per organ [Normalized: Cre+: 1.7(0.98-3.08); Cre-: (0.67(0.47-1.53)] and was enriched in $CD69^+$ $CD103^-$ [Cre+: 34.55%(18.58-47.55); Cre-: 16.7%(13.6-24.1)] and $CD49d^+$ $Ly6c^-$ [Cre+: 49.25%(46.6-56.05); Cre-: 21.8%(11.1-40.9)] cells. Altogether our results show that SUV39H1-deficiency leads to the accumulation of TRM-like $CD8^+$ $CD69^+$ $CD103^-$ cells in tissues at steady state. We then adoptively transferred naïve WT and SUV39H1-deficient OT-I $CD8^+$ T cells at a 1:1 ratio into lymphopenic RAG-KO mice. Between 33 and 35 days after transfer, the ratio of SUV39H1-KO to WT OT-I cells in non-lymphoid tissues raised from 1 up to 20 [small gut: 10.42(7.38-20.74); skin: 7.15(4.91-8.65); liver: 6.02(3.75-7.42); lung iv-:4.3(1.71-7.53)]. In lymphoid organs, the KO:WT ratio remained lower than 2 [spleen: 1.44(1.21-1.86); mLN: 1.5(1.32-1.63); blood: 0.96(0.86-1.02); iLN: 0.82(0.74-1.29)], indicating a clear bias of SUV39H1-defective T cells for homing to non-lymphoid tissues. Both percentages and numbers of $CD49d^+$ $Ly6c^-$ cells were higher among SUV39H1-deficient OT-I cells in all tested organs [lung iv-: KO: 69.4%(56.1-73.85), WT: 48.8%(27.4-56.75); liver: KO: 57.2%(48.9-61.7), WT: 14.4%(10.26-15.56); small gut: KO: 51.5%(34.3-62), WT: 22.3%(9.85-24.9); spleen: KO: 24.7%(22.95-36.25), WT:1.83(1.46-2.36)]. Increased proportions and numbers of SUV39H1 KO total OT-I $CD8^+$ T cells and $CD49d^+$ $Ly6c^-$ OT-I $CD8^+$ T cells in the iv- compartment of lungs were not a:ected by FTY720 treatment. These results show that SUV39H1 intrinsically restrains $CD8^+$ T cells homing to non-lymphoid tissues, inhibiting the accumulation of tissue resident, $CD49d^+$ $CD69^+$ cells. We next studied $CD8^+$ T cells in lungs of SUV39H1 KO mice infected with influenza. Iv- $CD49d^+$ $Ly6c^-$ $CD8^+$ T cells accumulated in the lungs of Cre+ mice 28 days after X31 infection [Normalized: Cre+: 1.45(1.21-1.67); Cre-: 1(0.825-1.18)]. Three days after a challenge infection with PR8, we observed an overall accumulation of iv- CD44+ CD8+ T cells per lung [Cre+: 6848(5187-14310); Cre-: 2505(1044-4935)], and increased numbers of $CD49d^+$ $Ly6c^-$ [Cre+: 571(285-803); Cre-: 88(48-158)] and $CD69^+$ $CD103^-$ [Cre+: 3149(1096-5564); Cre-: 1235(372-1525)] $CD8^+$ T cells among $CD44^+$ iv- cells in Cre+ mice previously infected with X31 strain. Finally, viral loads in the lungs of SUV39H1 Cre+ mice previously infected with X31 strain, were slightly (but significantly) reduced compared to WT littermates 3 days after PR8 secondary infection [-log10 normalized: 1.11(0.46-3.23); Cre-: 0.42(-0.1- 1.26)]. Altogether these results suggest that SUV39H1 prevents the accumulation of protective $CD49d^+$ $CD69^+$ $CD8^+$ TRM cells upon flu infection. To further characterize SUV39H1-KO T cells homing to tissues, we used CITE-seq analysis of sorted cells from lungs of RAG-KO mice after adoptive transfer of WT and SUV39H1-KO OT-I cells. Unsupervised clustering partitioned cells into 9 clusters based on their transcriptome at 0.8 resolution. We identified one cluster of cycling cells (cycling Mki67), four clusters of central memory cells (TCM CCR7 a-c and TCM Jun), three clusters of e:ector cells (TE$ Lgals3, TE$ CTL GzmA and TE$ CD49d), and one cluster of tissue resident memory cells (TRM CD69). Consistent with previous results, both TRM CD69 and TE$ CD49d clusters were among the most overrepresented in KO cells, compared to WT cells. Both Slingshot and Scvelo trajectory analyses suggested a di:erentiation trajectory starting with TCM CCR7 clusters, which di:erentiated into TE$ CD49d, which represented a branching point to the two final di:erentiation fates driving to either TE$ Lgasl3 and CTL GzmA or TRM CD69. These results suggest that TE$ CD49d cells are enriched in precursor cells that give rise to TRM CD69 cells. To formally test this, we transferred WT and SUV39H1-de:iceint naïve OT-I cells in a 1:1 ratio into B6 mice and then infected the mice with the flu strain X31 expressing the SIINFEKL peptide. SUV39H1-KO CD49d+ Ly6c-cells accumulated in the lungs from 9 days after infection [KO: 51.7%(46.53-52.68); WT: 15.9(15.36-22.7)]. WT and KO OTI cells from the iv- compartment of the lungs were FACS-sorted 9 days after X31-OVA infection and adoptively transferred intravenously at 1:1 ratio into recipient mice previously infected with X31-OVA. After 28 days, mice had higher proportions of SUV39H1-deficient OT-I cells, compared to

WT cells, among $CD69^+$ $CD103^-$ $CD8^+$ T cells in the iv- compartment of the lung [KO: 36.4(29.7-41.55); WT: 20.6(18.35-22.55)]. We conclude that SUV39H1-deficient $CD8^+$ T cells homing to the lungs during the acute phase of infection are enriched in precursors of CD69-expressing TRM, compared to WT cells. Finally, we observed that RAG-KO mice receiving SUV39H1-KO OT-I cells, delayed B16- OVA-Luc lung tumor growth significantly longer compared to mice bearing WT OT-I cells [Average radiance median at day 38pi: KO: 1053p/s/cm:–/sr(716.05-13020); WT: 24875 p/s/cm:–/sr(1090.9-89180)], suggesting a better control of lung tumor metastases by lung-homing SUV39H1-deficient CD8+ TRM cells. Conclusions: Our results suggest that SUV39H1 ablation increases tissue accumulation and di:erentiation of TRM precursors expressing high levels of the integrin CD49d. Also, the increase in tissue homing and the subsequent increased TRM cell di:erentiation in absence of SUV39H1 improves the e:icacy of memory immune responses in tissues, in both tumors and infection models. Inhibition of expression or function of SUV39H1 in T cells should help promote e:icient immune responses in the context of both adoptive and non-adoptive immunotherapies.

393. 129. **DYNAMICS OF THE B LINEAGE RESPONSE DURING INTRANASAL IMMUNIZATION WITH KLEBSIELLA PNEUMONIAE**

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Vaccines have long been crucial in protecting against various pathogens, with the generation and maintenance of lymphocytes in barrier tissues being key to their effectiveness. Recently, tissue-resident memory B cells (B_{RM}) have been identified as important in providing protection in infections like influenza. Our study aimed to characterize the kinetics, phenotype, and localization of B_{RM} following intranasal immunization with *Klebsiella pneumoniae* (Kp), a Gram-negative bacterium that causes atypical pneumonia for which no vaccine is available. In recent years, Kp has developed antibiotic resistance, making it a growing global health concern. C57Bl/6 mice were intranasally immunized on days 0 and 7 with heat-killed Kp. To distinguish circulating B cells from B_{RM}, we performed *in-vivo* labeling by injecting anti-CD45-A700 antibody intravenously 5 minutes prior to sacrifice, followed by i*n- vitro* labeling with anti-B200 FITC on lung cells. B cells began infiltrating the lungs by day 14 post- immunization and persisted as B_{RM} until at least day 60 post-immunization. During the memory phase, B_{RM} were characterized by the phenotype $B220^+$, IgD^{neg}, $CD69^{+/-}$, $CD38^+$, $PDL2^{+/-}$. Additionally, we identified IgA^+ plasmablasts that were reactive to Kp, as confirmed by ELISPOT. B_{RM} localization analysis revealed that by day 14 post-immunization, B cells were situated near blood and lymphatic vessels, while during the memory phase, they were primarily found near large airways and within the lung parenchyma. Our findings indicate that B_{RM} populations and Kp-specific IgA^+ plasmablasts are established in the lung following Kp immunization, and their roles in lung immunity will be further investigated.

394. 173. **AXL-MEDIATED SIGNALING CONTROLS CIGARETTE SMOKE LUNG INFLAMMATION AND COMPROMISES HOST IMMUNITY DURING PNEUMOCOCCAL PNEUMONIA**

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Smoking cigarette is a risk factor for pneumonia. Cigarette smoke (CS) induces inflammation on airways and impairs immune responses including the efferocytosis of apoptotic cells. The Axl and MerTk receptors mediate efferocytosis and simultaneously inhibit pro-inflammatory pathways. Here we investigated how Axl and MerTk signaling affects the outcome of *S. pneumoniae* infection in previously CS exposed mice. We first found high levels of nitric oxide in the fluids of the bronchoalveolar lavage (BALF) of CS-WT, CS-Axl-/-, and CS-$MerTk^{-/-}$ mice compared to AIR groups. BALFs from CS-$Axl^{-/-}$ mice, had an increased number of total airway cells, represented by resident AMs, IMs, neutrophils, and monocyte-derived AMs (moAMs) compared to CS-WT and CS- $MerTk^{-/-}$ mice. In addition, we found high

levels of TNF, IL-6, CXCL1 and low levels of IL-10 in CS-Axl$^{-/-}$ BALFs compared to CS-WT or CS-MerTk$^{-/-}$ mice. Additionally, CS-Axl$^{-/-}$ BALFs showed an increased number of late apoptotic and necrotic resident AMs and neutrophils compared to CS-WT and CS-MerTk$^{-/-}$ mice. After *S. pneumoniae* (SP) challenge, we observed lower mortality of CSSP-Axl$^{-/-}$ mice compared to CSSP-WT and CSSP-MerTk$^{-/-}$ mice. However, we observed SP translocation in the spleen of all CSSP groups and, surprisingly, lower bacterial CFU in BALFs from CSSP-Axl$^{-/-}$ mice compared to CS-MerTk$^{-/-}$ mice. We also found high levels of nitric oxide in the BALFs of CSSP-Axl$^{-/-}$ mice compared to CSSP-WT and CSSP-MerTk$^{-/-}$ mice. In addition, BALFs from CSSP-Axl$^{-/-}$ mice, had an increased number of total airway cells, represented by resident moAMs, and neutrophils compared to CSSP-WT and CSSP- MerTk$^{-/-}$ mice. Importantly, BALFs from CSSP-Axl$^{-/-}$ mice had more MHCII^{+} (M1-Like) moAMs compared to CSSP-MerTk$^{-/-}$ mice. Overall, our study suggest that Axl-mediated efferocytosis controls CS- induced lung inflammation influencing the host protection against SP.

395. 194. **A TRYPTOPHAN METABOLITE, INDOL-3-PROPIONIC ACID, REDUCES INFLAMMATION IN EXPERIMENTAL SMALL INTESTINAL ENTEROPATHY**

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Background: The microbiota and their metabolites has gained attention due to their potential therapeutic applications in gut inflammatory disorders. One metabolite produced by the microbiota from dietary tryptophan, the indole-3-propionic acid (IPA), has shown a beneficial effect on small intestine promoting intestinal homeostasis; however, the mechanisms underlying this effect remain unclear. To investigate the role of IPA in the intestinal mucosa, we have developed an experimental model of small intestine enteropathy that resembles celiac disease, one of the most common intestinal inflammatory diseases. **Objective:** To assess the potential beneficial effects of IPA administration on inflammatory reactions in the proximal small intestine. **Methods:** Eight week old C57BL/6 wild-type mice were treated daily via intragastric administration with IPA (0.5 mg/mouse) for four days. The p31-43 gliadin peptide (40 µg/mouse) or vehicle was administered on the fifth day and mice were sacrificed 16 hours later. Sections from proximal small intestine were used for histology (Villus height /Crypt depth (V/C) ratio analysis and counting intraepithelial lymphocytes (IELs) and goblet cells). Moreover, flow cytometry analysis was conducted on IELs isolated from intestinal fragments. **Results:** Administration of p31-43 peptide significantly decreased the V/C ratio (p=0,0180) The treatment with IPA was able to reverse the changes in the V/C ratio induced by p31-43 administration. IELs increased following p31-43 treatment, but this infiltration was diminished by IPA preconditioning. Flow cytometry analysis indicated an increase in γ/δ IELs number after p31-43 treatment, which was partially reversed by IPA treatment. Goblet cell numbers showed no significant differences across treatments. **Conclusion:** IPA preconditioning may modulate some inflammatory markers induced by the p31-43 peptide treatment. In particular, this effect seems to prevent partial villous atrophy, a hallmark of celiac disease. Further studies are needed to pinpoint the type of IELs that are modulated by both the p31-43 peptide and IPA.

396. 195. **SPATIAL AND PHENOTYPIC HETEROGENEITY OF INTESTINAL MACROPHAGES**

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Barrier mucosae are continuously exposed to sterile and non-sterile immune cues, thus a tight regulation of immune responses is needed. Mononuclear phagocytes, in particular, gut macrophages, play a critical role in immune defence and tissue homeostasis. They reside along the entire gastrointestinal tract and within all the layers of the

wall, however, their functional heterogeneity and ontogeny, as well as their microanatomical niche remain largely undescribed. To investigate the diversity, spatial distribution, origin, and function of gut macrophages, we employed a high-dimensional 100-plex Co-Detection by Indexing (CODEX) imaging approach onto gut sections of specific-pathogen-, germ-free wildtype mice, and a novel transgenic fate-mapping mouse model that allows detecting fetal and bone marrow-derived macrophages simultaneously. Unsupervised cell classification identified seven macrophage populations residing in unique cellular neighborhoods within the gut wall and along the longitudinal axis of the small and large intestines. Spatial transcriptomics (Xenium) showed similar macrophage clusters and revealed specific functional signatures associated with their microenvironment. Employing spectral flow cytometry, these macrophage populations were readily identifiable, as well as their ontogeny, characterized by being distinct and site-specific. Finally, to investigate the role of gut macrophages, we assessed the impact of acute dextran sulfate sodium (DSS)-induced colitis and altered diet (high-salt and high-fat) on the pool of intestinal macrophages throughout the gut, and observed different effects on the abundance of gut resident macrophages and their metabolism/function. Taken together, our findings demonstrate a hitherto undescribed regional heterogeneity in macrophage phenotype and ontogeny in the gastro-intestinal tract.

397. 216. **CD4+ T CELL FUNCTIONAL ADAPTATION TO MICROBIOTA MANIPULATION RESTORES INTESTINAL HOMEOSTASIS**

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Effector CD4+ T cells establish a close interplay with the microbiota, impacting the development of immune-mediated inflammatory diseases. However, some CD4+ T cells retain a degree of plasticity and can transition their phenotype, i.e. from effector to regulatory phenotypes. Still, whether this conversion occurs in an established immune-mediated disease in humans, which is a prerequisite for developing a targeted therapy, is unknown. Furthermore, mechanistic knowledge on how to manipulate CD4+ T cell plasticity *in vivo* remains elusive, hindering its translation to therapeutic strategies. Here, we performed longitudinal sampling of individuals with Inflammatory Bowel Diseases, i.e. Pouchitis, to study T cell plasticity during antibiotic-induced mucosal healing. We found that mucosal healing was associated with intestinal IL-17A+ clones acquiring a Foxp3+ phenotype. Using *Il17a* fate-mapping mice and an antibiotic-dependent relapsing IBD mouse model, we found that the Th17 cell phenotype continuously re-adapts to microbial changes. The conditional deletion of Foxp3 in Th17 cells curtailed antibiotic-induced mucosal healing, worsening disease relapse. Finally, microbiota-derived metabolites stabilized the regulatory Foxp3+ phenotype in former Th17 cells, sustaining intestinal homeostasis. Thus, we show that human and mouse CD4+ T cell plasticity adapts to changes in the microbial composition and metabolites, even under inflammatory conditions. We discovered that by manipulating the microbiota/metabolites axis, T cell plasticity can be shaped *in vivo* at will, i.e. towards a regulatory Foxp3+ profile, re-establishing immune homeostasis.

398. 262. **DECIPHERING THE ROLE OF GALECTIN-4 AND ITS GLYCOSIDIC LIGANDS IN INTESTINAL INFLAMMATION**

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Background: Galectin-4 (Gal-4) is expressed almost exclusively in the gastrointestinal tract of healthy individuals. This lectin presents different physiological roles including protein trafficking to the apical cell membrane of the enterocyte and stabilization of lipid rafts through interaction with glycoproteins and glycolipids. Moreover, galectin-4 enhances migration and proliferation of epithelial cells, suggesting a possible beneficial effect in wound healing; finally, this lectin has shown bactericidal activity against microorganisms expressing blood group antigens. **Objective:** This study aims to explore the expression and function of Galectin-4 (Gal-4) in the context of intestinal inflammation. **Methods:** Publicly available single-cell RNA sequencing (scRNAseq) data, both from mice and Ulcerative Colitis (UC) patients, was analyzed to investigate Gal-4 expression patterns. Spatial transcriptomics (ST) data from mice allowed Gal-4 expression along the gastrointestinal tract. Gal4 expression and binding was studied by confocal microscopy. Small intestinal organoids were isolated and stimulated with Gal-4 to assess its functional impact. To assess the *in vivo* implications of Gal4 dysregulation in intestinal inflammation, transgenic mice with Gal-4 depletion in the intestinal epithelium were subjected to dextran sulfate sodium (DSS)-induced colitis. **Results:** Transcriptomic analysis of UC patients indicated a downregulation of Gal4, primarily in epithelial cells. In vivo, Gal-4-depleted transgenic mice showed more pronounced weight loss compared to wild-type mice in DSS-induced colitis. By scRNAseq analysis, Gal4 is expressed in mice epithelial cells in both small intestine and colon. ST showed a uniform distribution with no significant variation along the distal-proximal or crypt-villus axes. Confocal microscopy confirmed Gal-4 expression in ileum and colon, and allowed to explore Gal-4 binding to the epithelium. Organoid assays demonstrated a reduction in organoid number upon Gal-4 stimulation. **Conclusion:** Our findings offer new insights into the spatial distribution and cell-specific roles of Gal-4, expanding our understanding of its role in intestinal inflammation.

399. 270. **STUDIES ON THE FUNCTIONAL ROLE OF TONSILLAR CD39HIGHCD73+ B CELL POPULATION**

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The tonsils are an immune protection at the entrance of the aerodigestive tract. Recently, we analyzed tonsillar mononuclear cells at different ages to explore the impact of aging and inflammation. We demonstrated a reduction in germinal center (GC) cells and identified an age-related increase in a B lymphocyte subpopulation expressing CD39 and CD73, cells with immunoregulatory activity. Here, we aim to further characterize this subpopulation.
We used flow cytometry for phenotypic assays and cell sorting to isolate $CD20^{+}CD39^{high}CD73^{+}$ and $CD20^{+}CD39^{int}CD73^{-}$ cells. These cells were cultured and stimulated with CpG and CD40L for 48 hours. To assess CD39 and CD73 localization and activity, we stained tonsillar tissue slides with Pb(NO3)2 and (NH4)2S and performed malachite green assays.
We found that $CD20^{+}CD39^{high}CD73^{+}$ cells exhibited higher expression of CD44, CD27, CD62L, IgD and IgM compared to $CD39^{int}CD73^{-}$ B cells. In contrast, the latter showed higher levels of

CD10, CD38 and CD21, markers of GC activity. Accordingly, over half (56,00%±17%) of the $CD20^{+}CD73^{-}CD39^{Int}$ cells were proliferating ($CD20^{+}CD73^{-}CD39^{Int}Ki\text{-}67^{+}$) at the time of the surgery, while most of the $CD20^{+}CD39^{high}CD73^{+}$ cells resulted $Ki\text{-}67^{-}$ (95,60%±1,96%). Topographically, we confirmed that CD39 and CD73 expression is predominantly localized in the follicular mantle and interfollicular zones through the staining of enzymatic activity on the biopsies.
ATP hydrolysis was measured in the sorted cells as an indicator of CD39 activity, revealing similar enzymatic activity between $CD20^{+}CD39^{high}CD73^{+}$ and $CD20^{+}CD39^{int}CD73^{-}$ cells. We hypothesize that the use of anti-CD39 and anti-CD73 antibodies during sorting might have blocked the catalytic sites of these enzymes, resulting in reduced activity in both subpopulations. Finally, when these cells were cultured and stimulated, $CD20^{+}CD39^{high}CD73^{+}$ subset exhibited negligible proliferation compared to $CD20^{+}CD39^{int}CD73^{-}$. Our current results indicate that the age-increasing CD20+CD39highCD73+ cell population resemble 'stem-cell-like' quiescent cells comprising a heterogeneous population of memory B cells, exhausted cells and plasmablasts.

400. 330. **LOW PH ACTS AS AN ENVIRONMENTAL CUE FOR THE HUMAN TISSUE RESIDENT MEMORY T CELL DIFFERENTIATION**

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T-resident-memory-cells (TRM) play a crucial role in defense against infections and in antitumor immunity. The mechanisms responsible for their differentiation are still partially understood. Our aim was to evaluate if low pH induces the differentiation of human CD8+ T cells in a TRM phenotype. CD8+T cells were obtained from healthy donors using magnetic microbeads and were activated with CD3/CD28 beads (48hs). Then were cultured 3 days at neutral (7.3) or low pH (6.5). The expression of CD69 and CD103 (phenotype of TRM) and IFN□, TNF and Granzime B production were determined by flow cytometry. TGF-β production was assessed using HEK-Blue TGF-β-reporter-cells. The exposure at low pH increase the percentage of CD69+/CD103+CD8+T cells vs neutral pH (24.8 ± 2.4 vs 4.0 ± 0.7, n=24 p<0.0001). Also, the percentage of CD103+TCD8+ cells was increased (44.0 ± 4.3 vs 16.7 ± 1.8, n=24 p<0.0001). CD4+T cells did not appreciably express CD103 (n=24). We observed that 4 h at low pH was sufficient to obtain CD8+CD103+T cells (n=3). Low pH treatments did not compromise viability (n=24). We observed an increase in the Granzime B production in CD8+CD103+ T cells compared to CD8+CD103- T cells in both pH 7.3 (MFI 138170.1 vs 103409.4, n=6 p<0.01) and 6.5 (MFI 117462.5 vs 66355.1, n=6 p<0.05). We didn't observe any difference in the production of IFN□ and TNF (n=5). The ability of low pH to induce the CD69+CD103+ phenotype in CD8+T cells was suppressed by treatment with ALK5 inhibitor (kinase associated with TGF-β receptor I) (n=3 p<0.05), suggesting an action mediated by autocrine production of TGF-β. Also, the production of bioactive TGF-β was increased in CD8+T cells exposure to low pH (n=6, p<0.05). Our findings identify a signal for the TRM differentiation program and may allow the generation of human TRM cells in vitro for translational applications.

401. 351. **THE DYNAMICS OF CD4+ T CELLS IN TONSILLAR FOLLICLES AND EPSTEIN-BARR VIRUS LATENT INFECTION IN ASYMPTOMATIC INDIVIDUALS**

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The tonsils are sites of intense germinal center (GC) activity, although we have recently shown that tonsillar GC B cells exhibit an age-dependent impairment. The relationship between the GC reaction and Epstein-Barr virus (EBV) in the asymptomatic host remains an unsolved puzzle. Here, we aim to characterize the evolution of GC and

extra-follicular (EF) CD4+ T cell subsets along with the expression of key viral proteins at different ages. We performed immunohistochemistry on tonsillar biopsies to detect the expression of the viral markers LMP1 and EBNA2. In addition, we used flow cytometry to analyze the proportion of cell subsets. We worked with a cohort of 25 individuals which included 15 children (6.2±2.8 years old), 5 teenagers (12.6±0.6 years old) and 5 adults (33.6±4.7 years old). Global CD4+ T cells proportion remains stable at all ages. We found a significant age-dependent decrease in the proportion of GC CD4+ T cells (r=-0.81, p<0.0001) together with an increment in the fraction of mantle CD4+ T cells (r=0.57, p=0.0029) as well as the EF CD4+ T cells (r=0.48, p=0.0162). When we analyzed LMP1 expression, we detected a significantly greater proportion of EF CD4+ T cells in LMP1 (-) samples (median=28.25, n=8) than in those of LMP1 (+) ones (median=16.80, n=17) (p=0.0104). We confirmed the same trend between LMP1 (+) and LMP1 (-) samples within each age group, hence excluding the co-founding factor of age. Interestingly, the same tendency applies when testing for EBNA2 expression (median=31.00, n=5 EBNA2 (-) vs median=17.80, n=20 EBNA2 (+); p=0.0439). LMP1 and EBNA2 expression was found in EF zones with the occasional appearance of EBNA2 in GC (2 patients). These findings indicate a correlation between the expression of viral proteins and the dynamics of CD4+ T subsets between follicular and extra-follicular zones beyond the ageing effect.

402. 385. INDUCTION OF TH-1 IMMUNE RESPONSE BY LACTICASEIBACILLUS PARACASEI SUBSP. PARACASEI CRL75 IN FOOD ALLERGY

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Background: Many lactic acid bacteria (LAB) have the ability to release and/or produce specific compounds with positive outcomes for human or animal host. Previously, we demonstrated that *Lacticaseibacillus (L.) paracasei* CRL75 has a potent immunostimulatory effect. **Objective:** to evaluate the ability of *L. paracasei* CRL75 to induce Th-1 immune response in a food allergy mouse model when administered in fermented milk or drinking water. **Methods:** Six-week-old BALB/c mice were divided into five groups: a) Control (C,n=8): non-sensitized animals received a conventional balanced diet and water *ad libitum*; b) OVA (n=8): animals sensitized to OVA by bi-weekly intraperitoneal injection; c) CRL75 (W-CRL75,n=8): OVA-sensitized animals receiving CRL75 in drinking water; d) Non-fermented milk (M,n=8): OVA-sensitized animals receiving non-fermented milk; e) Fermented milk (FM-CRL75,n=8): OVA-sensitized animals receiving CRL75 in fermented milk. Drinking water, non-fermented milk, and fermented milk were administered for 7 days prior to OVA challenge. Before allergy induction, three mice per group were sacrificed, spleens isolated, and splenocytes stimulated with OVA for 24 h. Unstimulated and LPS-stimulated cells were negative and positive controls, respectively. Cell culture supernatants were collected for cytokine measurement. Two weeks after second injection, intestinal allergy was induced by feeding egg white diet for 7 days. Sera samples were obtained to evaluate cytokines and OVA- specific antibodies. **Results:** In *ex vivo* assay, W-CRL75 and FM-CRL75 showed increased production of TNF-alpha, IFN-gamma, and IL-10 but significantly decreased IL-4 levels (pg/ml, C:148.8±21.5, OVA:627.6±25.2; M:556.6±10.7; W- CRL75:344.9±43.9; FM-CRL75:293.6±16.3, p<0.0001). In serum, CRL75 significantly decreased OVA-specific IgE (OD450nm, C:0.064±0.012; OVA:0.717±0.077; M:0.72±0.08; W-CRL75:0.449±0.029; FM CRL75:0.379±0.052, p<0.0001), but increased OVA-specific IgG1 and IgG2a compared to OVA group. In serum, both CRL75 administrations showed increased concentrations of IFN- gamma and IL-10, with significant reduction of IL-4. **Conclusion:** *L. paracasei* CRL75 is able to induce a Th-1 response by decreasing the Th-2 response, with reduced OVA-specific IgE and IL-4.

403. 390. ENVIRONMENTAL POLLUTION, ITS EFFECTS ON THE OCULAR MUCOSA AND TREATMENT WITH HUMAN AMNIOTIC MEMBRANE

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Particulate matter (PM) is an airborne pollutant

composed of particles of varying size and chemical composition. It originates from vehicle emissions and industrial activities. The ocular mucosa is directly exposed to PM, causing “urban conjunctivitis”, with symptoms and signs similar to dry eye syndrome. One of the differences between urban conjunctivitis and dry eye is that the latter presents tear hyperosmolarity. In the search for therapeutic alternatives, the human amniotic membrane (hAM) shows great potential in the treatment of immuno-inflammatory pathologies. The aim of this study was to investigate the effect of PM from a highly contaminated area of AMBA and hyperosmolarity on human conjunctival (IOBA-NHC) and corneal (HCLE) cells and to evaluate the ability of hAM to inmunomodulate these effects. PM of 2.5 µm was collected in Dock Sud, Avellaneda (2023-2024). Cell viability in the presence of PM, hyperosmolar saline solution, and hAM extracts was evaluated using the MTT assay. In addition the effect of hAM (100 µg/ml) on cell migration in the presence of PM (100 µg/ml) and under saline hyperosmolarity conditions (450 mOsm) was studied through the wound healing assay. Cytokine secretion was quantified with ELISA kits in the supernatants of cells stimulated with PM and under hyperosmolarity conditions and treated with hAM. Statistical analysis was performed using GraphPad Prism software, with one-way ANOVA and Tukey’s multiple comparison test (α=0.05). Under the conditions tested, no significant decrease in cell viability was found. Treatment with hAM modulated cytokine secretion in cells exposed to PM and saline hyperosmolarity and significantly increased wound closure speed in cell monolayers compared to untreated controls. The immunomodulatory and healing effects of hAM make it an interesting alternative for the treatment of ocular tissue affected by pollution (urban conjunctivitis) and hyperosmolarity (dry eye).

404. 403. **THE ARP2/3 COMPLEX INHIBITOR, ARPIN, PARTICIPATES IN EPITHELIAL BARRIER REGULATION DURING EXPERIMENTAL COLITIS**

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Background: Ulcerative Colitis (UC) is an inflammatory bowel disease characterized by colon mucosal inflammation that disrupts intestinal epithelial integrity and promotes dysbiosis, thus promoting proinflammatories pathways and leukocyte recruitment to the lamina propria. The actin cytoskeleton regulates the integrity of the epithelial barrier via tight and adherent junctions. The Arp2/3 complex, an F-actin branch nucleator, participates in junction formation and stabilization; however, functions of endogenous Arp2/3 complex inhibitors such as arpin in the regulation of epithelial barrier functions during colitis have not been studied. **Objective:** To evaluate whether arpin strengthens intestinal epithelial barrier integrity by regulating actin dynamics and maintaining apical junction complex architecture during experimental colitis. **Methods:** Arpin-KO mice were generated using Crispr-Cas9 genetic engineering. The dextran sulfate sodium (DSS)-induced colitis model was applied and the disease activity index was evaluated over 7 days. Colons were measured and prepared for histologic analysis. Lamina propria was digested for flow cytometry. Peripheral blood was extracted by cardiac puncture. **Results:** Arpin-KO mice started to exhibit disease symptoms significantly earlier compared to WT mice. Arpin KO-DSS mice showed an almost complete lost of crypt architecture in the distal colon, massive edema and leukocyte infiltration. Flow cytometry revealed significantly higher presence of CD4+ T cells and B lymphocytes in the lamina propria compared to controls. Preliminary data from arpin KO-DSS mice peripheral blood showed an increase of TLR4+ neutrophils compared to WT-DSS-mouse, suggesting bacterial translocation into the blood. **Conclusion:** The observed severe histological changes and clinical manifestations observed in colitic arpin-KO mice suggest that arpin has an important role in epithelial barrier regulation. Pharmacologic targeting of arpin could therefore be an interesting new strategy to relieve colitis symptoms in UC patients. Further studies are ongoing to reveal the underlying molecular mechanisms.

405. 415. **AXL-MEDIATED EFFEROCYTOSIS REGULATES LUNG INFLAMMATION AFTER ACUTE CIGARETTE SMOKE EXPOSURE**

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Smoking causes dependence and can lead to a

multitude of complications, such as various types of cancers and chronic obstructive pulmonary disease (COPD). Cigarette smoke (CS) exposure entails an inflammatory response characterized by intense cell apoptosis bringing into evidence the importance of studying the role of efferocytosis mediated by TAM receptors, Axl and MerTk, in controlling lung inflammation. This study aimed to characterize CS-induced inflammation in the lungs of wild-type (WT) and TAM deficient mice ($Axl^{-/-}$, $MerTk^{-/-}$). After four days of CS exposure, we observed a 100% survival rate and a slight weight loss compared to control groups. The concentration of nitrites, total protein, and bronchoalveolar fluid (BALF) turbidity increased across all CS groups compared to controls. We found high numbers of total BALF cells in CS-$Axl^{-/-}$ and CS-$MerTk^{-/-}$ groups compared to controls, and even higher numbers in CS-$Axl^{-/-}$ and CS-$MerTk^{-/-}$ compared to CS-WT mice. Across CS groups, we observed an increase in alveolar macrophage (AMs) numbers of CS-$Axl^{-/-}$ compared to CS-WT and CS-$MerTk^{-/-}$ groups. Although CS groups showed higher numbers of monocyte-derived AMs, interstitial macrophages, and neutrophils compared to its control groups, CS-$Axl^{-/-}$ mice showed an even higher numbers compared to CS-WT or CS-$MerTk^{-/-}$. Lastly, we found high levels of TNF, IL-6, and CXCL1 in CS-WT and CS-$Axl^{-/-}$ compared to its control groups and even higher levels in CS-$Axl^{-/-}$ compared to CS-WT and CS-$MerTk^{-/-}$. Altogether, this data suggests that efferocytosis mediated by the Axl receptor plays an important role in regulating lung inflammation after acute CS exposure.

406. 416. **AMARANTH PEPTIDES FROM FUNCTIONAL FOOD EXHIBIT ANTI-INFLAMMATORY PROPERTIES MICAELA**

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Amaranth, a pseudocereal known for its high-quality protein content to modulate intestinal inflammation and PepT1 expression. Intestinal inflammation disrupts the epithelial barrier and attracts immune cells. The transporter PepT1 playing a role in this process. This study aimed to assess whether a functional food derived from amaranth (FF) or the SSEDIKE Amaranth peptide (AP) could modulate intestinal inflammation and PepT1 expression. We analyzed PepT1-expression in Caco-2 cells by confocal microscopy and the differential expression when cells were stimulated with proinflammatory cytokines (TNFα). PepT1-expression was also evaluated in IBD patients colon biopsies by quantitative PCR. Finally, we induced colitis in a mouse model using TNBS and treated them orally with AP, FF or FF+AP. We monitored the corporal weight, calculated the disease activity index (DAI) and analyzed the colonic inflammatory response. Results showed that PepT1 expression in Caco-2 cells was enhanced under inflammatory conditions ($p<0,05$). Inflamed colonic areas in IBD patients also exhibited significant up-regulation of PepT1 mRNA compared with non- inflamed paired samples ($p<0.05$). Finally, we found in mice that AP-treatment ameliorated the TNBS-induced colitis with a reduced DAI and a significantly decreased expression and production of proinflammatory cytokines (mRNA FI, Ccl20: 2.1±0.2vs4.5±0.3; TNF: 2.3±0.45vs2±0.7; and IFN-γ protein levels: 326±57vs748±34 pg ml^{-1},$p<0.01$) compared to untreated colitis mice. Remarkably, our findings showed a significant down-modulation of PepT1 transcripts in AP-treated mice ($p<0.05$). Conversely, FF-treatment failed to modulate colitis, whereas AP-supplementation reduced intestinal inflammation, evidenced by the macroscopic characteristics of the colon and the histology. In conclusion, our findings indicate that the amaranth peptide SSEDIKE exerted an anti-inflammatory effect through the reduction of PepT1 expression. Furthermore, the FF, which does not exert an anti-inflammatory effect per se, should be supplemented with the peptide. These findings may pave the way to develop a functional food based on the use of a peptide of Amaranth.

407. 425. **IMPACT ON THE IMMUNE RESPONSE AGAINST GASTROINTESTINAL INFECTION AFTER A PROPHYLACTIC PULMONARY IMMUNIZATION WITH INACTIVATED SARS-COV-2**

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The interactions between host-pathogens can modulate a functionality of local cells, immune system, and influence on development of diseases. Studies have been shown to impact of this interactions goes far beyond the local environment. There is across talk between the mucosal site in our body, one of these examples is the communication with gut and lung, too named axis gut-lung, and to influence the immune response of both of organs. Although the mechanisms of this cross talk are not yet well elucidated, studies indicate this occurs through the transit of cells and chemical messengers produced directly by microorganisms, and by the immune response that they direct. These chemical messengers are carried via the blood or lymphatic pathways to regulate immune system functions throughout the body. We evaluated gut-lung axis crosstalk in C57BL/6 mice immunized with inactivated SARS-CoV-2 virus and challenged these animals with *Y. pseudotuberculosis* bacteria. Our hypothesis is how previous pulmonary immunization alters the immune response to gastrointestinal infection. Preliminarydata showed, although without statistical significance, animals challenged with bacteria recovered better body weight after infection compared to immunized and infected group, this show that SARS-CoV-2 antigen influences protection against gastrointestinal infection. The analysis of pulmonary inflammatory cells corroborates that data, a decrease in populations of cells such as monocytes and macrophages were observed in the same groups without a statistical difference; however, the IL-6 cytokine production by these cells decreases statistically. Another data observed was the statistical decrease of populations of T $CD4^+$ and T γδ lymphocytes in lungs of this group. The analyzes have pointed to a possible migration of inflammatory cells from lungs to intestines of immunized and challenged group.

408. 435. **ENTEROAGGREGATIVE ESCHERICHIA COLI (EAEC) AND SHIGA TOXIN-PRODUCING ENTEROHEMORRHAGIC ESCHERICHIA COLI (EHEC) CO-INFECTION PROLONG BACTERIAL SHEDDING IN VIVO**

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Hemolytic Uremic Syndrome (HUS) is a clinical entity characterized by the presence of hemolytic anemia, thrombocytopenia and acute renal failure. The direct cause of kidney damage in typical HUS is Stx. In recent years, ANLIS-Malbrán has detected an association of EAEC isolates with cases of HUS and bloody diarrhea in children. The aim of this study is to analyze whether infection with EAEC strains favors co-infection with EHEC strains and/or whether the inflammatory state of the intestine observed during infections with EAEC strains predisposes to a worse outcome compared to EHEC infections, without affecting the degree of colonization of these strains. To achieve this objective, an in vivo model of the C57BL/6 strain is used in mice at weaning age (17-19 days of age). Our previous in vitro studies demonstrated that preinfection with EAEC increased EHEC adherence ($p<0.005$). In addition, EAEC induced morphological changes and cell detachment in Caco-2. Likewise, co-infection increased il6 transcription ($p<0.0001$), produced an additive effect in ccl20 ($p<0.0001$) and enhanced il1o transcription ($p<0.0001$) compared to monoinfections; and reduced TGFo transcription in both monoinfections and co-infection compared to baseline ($p<0.0005$). When infecting c57BL/6 mice with EAEC, an increase in intestinal permeability was observed after 8 days ($p<0.05$), in addition to this, a decrease in the weight of mice co-infected with EAEC:EHEC was observed from 48 hours ($p<:0.05$), which was correlated with a significant decrease in feed intake ($p<:0.05$). Regarding the bacterial persistence in the intestine of mice inoculated on day 8 post-infection with EHEC, it was observed that animals co-infected with EHEC 125/99 and EAEC 118/19 had a higher number of CFU/g fecal matter of both strains compared to monoinfected animals ($p<0.0001$),(***$p<0.001$),($p<0.05$). On the other hand, EHEC 125/99 was not detected in the fe-

cal matter of monoinfected mice. In addition, an increase in plasma urea levels at 72 hours was observed in 3 out of 5 co-infected mice, together with an increase in the percentage of transient PMN that at 72 hours returns to baseline, in plasma of co-infected mice high levels of IgG antibody for EHEC compared to levels of EAEC igG antibodies ($p<0.0001$), one of 5 co-infected mice did not present high levels of IgG EHEC, in addition to this, he presented a progressive decrease in weight, urea of 93 mg/dl and leukocytosis with neutrophilia greater than 30%.
In conclusion, these results show that pre-infection with EAEC 118/19 and subsequent infection with EHEC 125/99 of mice at weaning age determines a worse overall outcome than that observed with mice infected only with EHEC 125/99 or EAEC 118/19, observed as weight loss, lower feed intake, increased plasma urea concentration and increased percentage of PMN. and changes in intestinal permeability: these changes could be contributing to their access of toxin to the systemic compartment.

409. 452. EVALUATION OF THE DEXTRAN SODIUM SULFATE (DSS) COLITIS MODEL. DIFFERENTIAL EFFECTS IN THE PROXIMAL SMALL INTESTINE AND COLON

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Background: Dextran sodium sulfate (DSS) is commonly used to induce colitis in mouse models. Although the precise molecular mechanisms remain unclear, DSS-induced inflammation and epithelial damage are thought to result from alterations in the mucosal barrier. However, there is limited information regarding its effects on the small intestine. **Objective:** To characterize the inflammation and tissue damage induced by DSS treatment in the mucosa of the proximal small intestine. **Methods:** Eight-week-old C57BL/6 mice were administered DSS at concentrations of 2.5% or 3% in drinking water for five days, followed by three days of water alone. Proximal small intestine and colon tissues were collected for histological analysis. The number of goblet cells was quantified in tissues stained with Alcian blue. Caspase-1, an indicator of inflammasome activation, was assessed via western blotting of total protein extracts from both the small intestine and colon. **Results:** Histological analysis of the proximal small intestine showed no significant changes in the villus height-to-crypt depth (V/C) ratio (the most common parameter to assess histological damage). However, there was a trend towards an increased number of intraepithelial lymphocytes in DSS-treated mice at both DSS concentrations tested. Unlike the decreased observed in the colon, the number of goblet cells in the small intestine remained unchanged. Western blot analysis demonstrated that DSS treatment activated the inflammasome in both the small intestine and colon, with caspase-1 activation evident in mice treated even at the lower DSS dosis. **Conclusion:** The effects of DSS treatment exhibit significant differences between the small intestine and colon. Despite inflammasome activation, no histological damage, evidenced by the V/C ratio and goblet cell count, was observed in the small intestine. This suggests the presence of distinct protective and repairing mechanisms operating in different segments of the intestinal tract.

410. 456. UPREGULATION OF MICRORNA-21 IN CHRONIC INTESTINAL INFLAMMATION MAY CONTRIBUTE TO FIBROSIS AND TISSUE DAMAGE THROUGH FIBROBLAST ACTIVATION PROTEIN (FAP) INDUCTION

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Fibroblasts are key stromal cells in Inflammatory Bowel Disease (IBD) contributing to fibrosis and colorectal cancer (CRC) development. Fibroblast activation protein (FAP) and microRNA-21 (miR-21) are markedly increased in several tumours. We aimed to explore FAP and miR-21 in fibroblasts from inflamed colonic mucosa to understand their potential contribution to tissue damage. Colonic biopsies and surgical samples were taken from IBD(n=40), CRC(n=8) and healthy individuals (HC)(n=4) and fibroblast primary cultures were established. FAP and α-SMA were evaluated by immunofluorescence (IF) and immunohistochemistry using tissue microarrays. MiR-21, *FAP* and *ACTA2* were quantified by qPCR in biopsies. Exosomes were enriched from fibroblast culture supernatants by ultracentrifugation (visualised by atomic force microscopy and western blot), and miR-21 expression was analysed. *In vitro* induction of FAP was evaluated in fibroblasts by IF, after stimulation with hrTGF-β or exosomal fractions. RNA samples from fibroblast lines were used for paired end RNA-seq. Raw sequencing reads were trimmed and then clean reads were mapped to the reference genome using HISAT2. Gene expression levels were estimated using Stringtie software with default settings. Differential gene expression analysis was performed using DESeq2 with default settings. MiR-21, *FAP* and *ACTA2* were overexpressed the in inflamed mucosa compared with HC and non-inflamed mucosa from IBD and CRC patients (**p<0,01, ***p<0,001, respectively). Both proteins were increased in the stroma of inflamed areas from the same patient samples (*p<0,05). hrTGF-β and miR-21-rich-exosomal fractions induced FAP expression in fibroblast primary cultures *in vitro* in comparison with unstimulated fibroblast (mean ± SEM; *p<0,05).
MiR-21 overexpression in chronic inflamed mucosa may induce fibroblasts activation pathways, contributing to tissue remodelling and damage. Unveiling the role of miRNAs and proteins in IBD and CRC development, may improve preventive and therapeutic strategies.

411. 492. UNVEILING THE INFLUENCE OF ORAL DYSBIOSIS IN THE DEVELOPMENT OF SQUAMOUS CELL CARCINOMA

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Background: Oral dysbiosis triggers a chronic inflammatory process to destroy periodontal support issues and systemic low-grade inflammation. This low-grade inflammation can contribute to oral cancer development. Despite advances in diagnosis and treatment of oral squamous cell carcinoma (OSCC) this disease starts with a pre-malignant lesion leukoplakia. Oral dysbiosis in patients can promote inflammation by modulatory molecules, which favors carcinogenesis in OSCC. **Objectives:** To determine the influence of oral dysbiosis in OSCC. **Methods:** Here, we evaluated tissues of health individuals and periodontal patients with leukoplakia or OSCC through RNA-Seq analysis the different signatures in long non-coding RNAs, gene ontology and the gene expression of immunomodulatory molecules involved in carcinogenesis. Also, we evaluated the presence of immune cells populations in primary tumor. We performed a meticulous transcriptomic analysis comparing leukoplakia and primary tumor samples of OSCC. **Results:** A dominance of biological processes intricately linked to bacterial presence and the heightened expression of patterns recognition receptors (PRRs) such as NOD2, TLR2, and TLR4 and high expression of receptors associated to recognition of secondary metabolites derived from microbiota such as short chain fatty acids (SCFAs) exclusively within OSCC specimens and diverging from their leukoplakia counterparts. We determinate malignant transformation due immunomodulatory molecules and immune population in tumor samples. In addition, we obtained a difference in non-coding RNA profile in samples of health individuals and samples from patients with leukoplakia or OSCC, which correlates with the different immunomodulatory molecules. **Conclusions:** These revelatory findings underscore a profound nexus between the microbiota and malignancy. It's

plausible that this symbiotic relationship thrives due to the compromised barrier function inherent in OSCC considering their presence, facilitating the translocation of bacteria or their metabolites into the epithelium. This discovery underscores the potential importance of investigating the transcriptional signature of non-coding RNAs, opening new avenues for understanding and potentially targeting this critical process in cancer progression.

412. 495. AXL-MEDIATED EFFEROCYTOSIS MODULATES IMMUNE CELL POPULATIONS OF THE PULMONARY MUCOSA IN MICE EXPOSED TO CIGARETTE SMOKE AND CHALLENGED WITH STREPTOCOCCUS PNEUMONIAE

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Cigarette smoke (CS) is a leading cause of death worldwide. CS-induced inflammation leads to cell death and accumulation due to defects on efferocytosis and it is a strong risk factor for pneumococcal infections, such as by *Streptococcus pneumoniae* (SP). The aim of this study was to evaluate the role of efferocytosis mediated by Axl and MerTk in modulating immune cell populations in the lungs of mice exposed to CS (4 days) and challenged with SP. Three days after SP infection, we found that CSSP-MerTk$^{-/-}$ group had a 50% decrease in survival compared to CSSP-WT and CSSP-Axl$^{-/-}$. All infected groups lost weight, especially CSSP-MerTk$^{-/-}$. Bronchoalveolar lavage fluids (BALFs) from CSSP-MerTk$^{-/-}$ had higher CFU counts compared to AIRSP-group. CSSP-WT mice showed increased BALF turbidity and total protein concentration compared to CSSP-Axl$^{-/-}$ or CSSP-MerTk$^{-/-}$ whereas BALFs from CSSP-Axl$^{-/-}$ showed higher nitrites concentration compared to CSSP-WT and CSSP-MerTk$^{-/-}$. We observed increased numbers of BALF total cells in CSSP-Axl$^{-/-}$ compared to CSSP-WT and CSSP-MerTk$^{-/-}$ groups. In addition, we found decreased numbers of alveolar macrophages (AMs) in BALFs from CSSP-WT and CSSP-Axl$^{-/-}$ compared to AIRSP groups; but high numbers of monocyte-derived AMs and neutrophils in the BALFs of CSSP-Axl$^{-/-}$ compared to CSSP-WT and CSSP-MerTk$^{-/-}$ groups. Lastly, BALFs from CSSP-Axl$^{-/-}$ or MerTk$^{-/-}$ had high levels of IL-6 or IL-10, respectively, compared to AIRSP groups. These results suggest that Axl receptor controls immune cell populations dynamic in the airways of CS exposed mice, which increase resistant to SP infection.

413. 501. THE ROLE OF MERTK RECEPTOR IN EXPERIMENTAL PERIODONTITIS DEVELOPMENT IN MICE

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Background: The oral cavity harbors the second largest microbial community in the human body. The translocation of oral bacteria to systemic sites can occur by oro- intestinal translocation through swallowing. The loss of colonization resistance and subsequent proliferation of pathobionts and oro-digestive translocation may be influenced by many immunological processes. Susceptible mice developed mucosal inflammation due to the lack of the MerTk tyrosine kinase receptor, which plays a role in efferocytosis. Objectives: We hypothesize that efferocytosis could be a mechanism contributing to the susceptibility of individuals to increased inflammation in the mouth and the subsequent disruption of oral-intestinal balance. Methods: Wild-type (WT) and MerTk knockout mice were used to induce the ligature-induced experimental periodontitis (Perio) that consists of the placement of a silk ligature around the second molar of each mandible for 15 days. The mandible, gingiva and cervical lymph nodes were collected for further analysis. Results: The alveolar bone loss of the Perio group increased compared to Sham mice in the WT (3,45mm vs 1,80 mm) and MerTk groups (4,43mm vs 2,19 mm). However, no significant differences were seen between the WT and knockout mice. The levels of Myeloperoxidase, Tumor Necrosis Factor, and Interleukin-1β in the gingiva likewise rose in a manner consistent with the pattern of bone loss. Concerning the cell populations in the cervical lymph node, the WT mice exhibited a higher number of CD4+ cells between the DP and Sham groups. However, in the DP-Mer group, there was a lower cell count compared to all other groups. Both Mer groups had a lower abundance of Th17 cells in comparison to WT mice. Conclusion: The data indicate that the MerTk receptor is not the sole requirement for the development of periodontitis. However, the absence of this receptor may disrupt the lymphoid compartments, which is important for tolerance.

414. 521. **MERTK RECEPTOR DO NOT CONTROL INTESTINAL INFLAMMATION DURING EXPERIMENTAL COPD**

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Introduction: Inhaling tobacco smoke causes Chronic Obstructive Pulmonary Disease (COPD), a lung disease that also affects the intestines, causing inflammation, increased permeability, impaired food digestion, and gut disbiosis. Understanding how immune responses are controlled during COPD may help in the management of intestinal diseases in smokers. Efferocytosis mediated by TAM receptors during lung inflammatory diseases has been explored, but its impact on the lung-gut axis during COPD has not been addressed yet. Here, we investigated the role of MerTk-mediated efferocytosis in the susceptibility of COPD individuals to intestinal inflammation. Methods: We exposed WT and MerTk$^{-/-}$ C57/BL6 to either room air (RA) or nine cigarrettes per day (CS), five times a week, for three months. We harvested the colons for *lamina propria* immune cells and cytokine analyses. Results: We found a decreased frequency of colonic macrophages and monocytes of at least 2-fold in the WT-CS group. We found similar neutrophil frequencies in both the WT and MerTk CS groups. Regardless of air or smoke cigarrette exposure, MerTk knockout mice had less TNF in the colonic supernatant compared to WT, respectively. Finally, the amounts of IL-1β and IL-17A were also negligible in MerTk animals vs. WT and between air and COPD conditions. Conclusion: Our preliminary data indicate that MerTk may not be involved in controlling immune responses in the intestines during COPD.

415. 563. **ROLE OF TGF-BETA 3 AND TCR-GAMMA DELTA T CELLS IN ORAL TOLERANCE**

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Dietary antigens drive the differentiation of peripheral regulatory T cells (pTreg) in mesenteric lymph nodes (mLN) through a TGF-β-dependent mechanism, a process known as oral tolerance. This ensures immune unresponsiveness to ingested proteins and maintains immune system balance. Although mammals have three TGF-β isoforms (TGF-β1, 2, and 3), their distinct functions, particularly in generating regulatory T cells and oral tolerance, are not fully understood. Given the high expression of TGF-β3 in barrier tissues, including the small intestine, this study investigated its role in oral tolerance. Oral tolerance to ovalbumin (OVA) was induced in C57BL/6 mice by adding 1% OVA to their drinking water. Mice were then immunized with OVA in CFA, and anti-OVA IgG levels and footpad thickness were measured. TGF-β3 was neutralized with an isoform-specific antibody or through genetic deletion of Tgfb3 in various cell types. Neutralizing TGF-β3 during OVA exposure abolished oral tolerance, while TGF-β1 neutralization had no effect. Inducible deletion of Tgfb3 (Tamoxifen^{CRE}) confirmed its necessity for oral tolerance, with deletion in hematopoietic cells (i-Vav^{CRE}) also impairing tolerance. Deletion in dendritic cells (CD11c^{CRE}) and TCR-α/β T cells (CD4^{CRE}) did not affect tolerance. Deletion of *Tgfb3* in TCR-γ/δ T cells partially compromised tolerance. Evaluation of Tregs in

TGF-β3-deficient mice showed reduced CD103 and α4β7 expression and lower Foxp3 levels in the small intestine lamina propria, suggesting a critical role for TGF-β3 in supporting Treg function in the gut. These findings were consistent in an antigen-specific system with OT-II. CD45.1. *Foxp3*^{GFP} cells.
Additionally, Tregs generated with TGF-β3 showed enhanced metabolic fitness and migration into the lamina propria. TGF-β3 production by TCR-γ/δ T cells and other hematopoietic cells is essential for oral tolerance, imprinting pTregs with necessary metabolic and adhesion profiles. This understanding could inform new therapies for food allergies, inflammatory bowel disease, and other autoimmune conditions.

416. 578. **ORAL TOLERANCE IS ESTABLISHED GRADUALLY**

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Oral tolerance inactivates CD4 T cells specific to dietary antigens through different mechanisms that include deletion, anergy, and acquisition of regulatory capacities. The factors that influence these different outcomes are not known. The aim of this work was to analyze the role of antigen-associated factors (e.g., concentration, frequency, TCR affinity) in determining CD4 T cell fates during oral tolerance induction. For this purpose, we adoptively transferred naïve CD45.1+ FoxP3GFP OT-II CD4 T cells and fed the mice with different regimes of the model antigen ovalbumin (OVA) that is recognized by OT- II cells with high affinity. When a single dose of OVA was fed to the mice, FoxP3 expression, upregulation of anergy- associated molecules, and deletion were observed in different levels. OVA induced an early bout of OT-II proliferation that was accompanied by upregulation of FoxP3 that peaked 48 hours after OVA administration. At later time points, the proportion of FoxP3+ cells decreased and anergic cells gradually became the dominant population. Cellular death followed until adoptively transferred cells were not detected. Repetitive administration of OVA induced the expression of anergy-associated surface molecules (e.g., Lag-3, neuropilin) and transcription factors (e.g., TOX, Egr2, Helios) in a dose- dependent manner suggesting that continuous exposure to cognate antigen induces a more profound CD4 T cell inactivation. *In vitro* examination of tolerogenic fates using different affinity ligands, showed that weak TCR activation is associated with the development of anergy. Collectively, these results indicate that induction of oral tolerance is established through a successive process where CD4 T cells acquire FoxP3 expression in a short-lived manner, progress into an anergic phenotype and are ultimately deleted. These results denote peripheral tolerance to oral antigens is a dynamic process, in which T cells modulate their function according to antigen persistence and affinity.

417. 586. **EVALUATION OF SEMINAL FLUID CAPACITY TO PROMOTE TRM PHENOTYPE IN MICE**

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Semen contains cytokines and metabolites with immunomodulatory properties. While the vaginal environment is typically restrictive to memory T cell entry, this restriction can be overcome during inflammation, allowing the establishment of resident memory T cells (TRM). We hypothesize that semen facilitates the recruitment of memory T cells and promotes their differentiation into a TRM phenotype. We observed that seminal vesicle fluid (SVF) enhances the protective effects of intravaginal and systemic (Prime-Pull) vaccination with inactivated HSV-2 (10^4-10^6 PFU/mouse). Here, we explore the mechanisms behind this process through two main questions: Can semen, in the absence of inflammation, recruit lymphocytes and promote their differentiation into a TRM phenotype? Can SVF promote the TRM phenotype in an already established memory population? In the first set of experiments, naive C57b/6-gbT mice (6 weeks old) were treated with SVF (or PBS) intravaginally and analyzed at weeks one, two, and three by flow cytometry. No significant differences in CD8, CD4, or total lymphocyte counts were observed between the groups (n=3 independent experiments, 5 individuals per group). To address the second question, naive mice were challenged intravaginally with a non-infective dose (10^4 PFU/mouse) of HSV-2 to generate a memory population in the vaginal

epithelium. Twenty-five days later, the mice were treated with SVF intravaginally, and two weeks after treatment, vaginal cells were analyzed by flow cytometry. Our findings showed that SVF increased lymphocyte numbers in the vaginal lumen (p=0.0286), with elevated expression of CD103+CD69+ cells (p=0.0143) (n=1 experiment, 5 individuals per group). These preliminary findings suggest that while SVF alone cannot recruit immune cells, it may promote the TRM phenotype within an established memory population, indicating the need for continued research.

418. 617. **BUTYRATE'S DUAL ROLE IN SPONDYLOARTHRITIS: FROM GUT GUARDIAN TO INFLAMMATORY TRIGGER**

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5.Gastroadvanced S.A

Background Spondyloarthritis (SpA) has been linked to dysbiosis, which disrupts the production of short-chain fatty acids (SCFAs), potentially influencing humoral immune responses and disease activity. Previous findings suggest that variations in SIgA levels may be associated with fluctuations in disease activity. This study aims to explore the relationship between fecal SCFA concentrations, serum SIgA levels, and disease activity indices in SpA patients Methods: 24 adults were assessed, including 12 with SpA without IBD; 7 with gastrointestinal symptoms (SpA-GI) and 5 patients with SpA without gastrointestinal manifestations (SpA-non-GI), 6 healthy subjects serving as the eubiosis group (HS) and 6 with IBD. Evaluations included gastrointestinal symptoms, BASDAI/ASASCRP indices, fecal calprotectin and serum secretory IgA (SIgA) levels. Results: SpA-GI patients exhibited elevated SIgA levels ($p < 0.001$), while SpA-NG patients showed no significant differences in inflammatory markers. The IBD group had elevated ESR, CRP, and SIgA ($p = 0.010$), reflecting intense inflammation. SCFAs levels were lower in SpA-GI and IBD groups compared to HC, indicating potential microbial dysbiosis, particularly low butyrate in IBD ($p < 0.015$). In SpA-NG, butyrate levels inversely correlated with serum SIgA (CC: -0.90, $p = 0.037$) and directly with disease activity indices ($p < 0.005$). Conclusion: Butyrate, a short-chain fatty acid, typically promotes gut health by enhancing T regulatory (Treg) cells and inhibiting the NF-κB pathway. However, in SpA, it may also have pro-inflammatory effects. This dual role is mediated by butyrate's interaction with G-protein coupled receptors (GPCRs) and its inhibition of histone deacetylases (HDACs), which can promote the expression IL-17. These mechanisms may account for the inverse relationship between butyrate levels and SIgA observed in SpA-NG patients, alongside its correlation with disease activity. Butyrate plays a complex immunological role in SpA, balancing between anti-inflammatory and pro-inflammatory effects depending on the gut environment.

419. 625. **MACROPHAGE FUNCTIONS ARE ALTERED BY HYPERTHERMIA**

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Fever is one of the five cardinal signs of inflammation. The immune system recognizes pyrogenic agents, which cause an increase in body temperature. Macrophages are the main cells responsible for pathogen clearance by phagocytosis, and we believe that temperature changes during infections can affect their function. Here, we investigated the impact of temperature on macrophage functional modulation. For functional analyses, we stimulated RAW 264.7 macrophages with lipopolysaccharide (LPS) with the bacteria *Streptococcus pneumoniae* at different temperatures. We found that macrophages pre-stimulated with LPS were able to uptake more *S.p* colonies at 37°C than at 39°C. However, after 6 hours of infection, macrophages pre-stimulated with LPS had a higher microbicidal effect at 39°C

than at 37°C. Next, we investigated possible mechanisms that may support these differences. We found that 6 and 24 hours after infection, LPS- stimulated macrophages at 39°C produced fewer nitrites, IL-6, and TNF-a than LPS- stimulated macrophages at 37°C. However, increasing the temperature to 39°C had no effect on the release of IL-10 in LPS-stimulated macrophages infected with *S.p*. Our data indicate that higher temperatures, as occurs in fever settings, inhibit several inflammatory functions of macrophages but do not interfere with their microbicidal ability.

420. 668. **REGULATION OF SIGIRR RECEPTOR IN NATURAL KILLER (NK) CELLS BY IL-18 AND IL-37 IN PATIENTS WITH ULCERATIVE COLITIS**

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Ulcerative colitis (UC) is an inflammatory disease affecting colon and rectum mucosa, linked to dysregulation of the immune system. Natural killer (NK) cells participate in proinflammatory responses through secretion of cytokines such as IFN-gamma and TNFa, and also generate anti-inflammatory effects by releasing IL-10, modulating the immune response and favoring suppression of inflammation. Regulation is mediated by the SIGIRR receptor, which controls the inflammatory (IL-18) and anti-inflammatory (IL-37) pathways. SIGIRR expression has been found to be reduced in the intestinal mucosa of UC patients compared to healthy individuals, but this has not been studied in peripheral blood NK cells. Chronic inflammation in UC could alter NK cells response to IL-18 and IL-37 stimuli compared to healthy individuals. This study aimed to evaluate the SIGIRR receptor content and regulation by IL-18 and IL-37 on peripheral blood NK cells from UC patients. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples of healthy and Mayo 2 UC patients by ficoll solution. PBMCs were cultured and treated with IL-18 and IL-37 for 18 hours. SIGIRR receptor content was observed through flow cytometry. Baseline SIGIRR content was slightly higher in UC compared to healthy patients (12.1% vs. 10.8%). Inflammatory stimulation with IL-18 increased SIGIRR content in healthy and UC patients (19.3% vs. 13.6%). Anti-inflammatory stimulation with IL-37 increased this in both groups; higher in healthy patients (15.5% vs. 8.02%). Stimulation with both cytokines generated an increase in receptor content; significantly higher in healthy patients (28.2% vs. 16.2%). These results indicate that low content and limited ability to increase SIGIRR receptor levels in NK cells after IL-37 stimulation could be associated to reduced SIGIRR presence in cells from UC patients compared to healthy individuals. This suggests a possible alteration in the inflammation control mechanisms typical of UC.

421. 688. **KLUYVEROMYCES MARXIANUS CIDCA 8154 ALLEVIATES IRINOTECAN INDUCED MUCOSITIS WITHOUT AFFECTING ANTITUMOR ACTIVITY**

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Background: Irinotecan is a chemotherapeutic agent commonly used in colorectal cancer treatment. However, its administration is associated to gastrointestinal side effects which usually lead to treatment discontinuation and adverse impact in disease outcome. Previous studies suggest that the probiotic yeast *Kluyveromyces marxianus* CIDCA 8154 (Km8154) is able to mitigate irinotecan induced intestinal mucositis. **Objectives**: This study aims to elucidate the intestinal protective mechanisms of Km8154 and evaluate its effect on irinotecan antitumor activity using a tumour-bearing mouse model. **Methods**: CACO-2 intestinal epithelial cells were treated with irinotecan and supplemented with Km8154 and gene expression was assessed via qPCR after 6h of treatment. BALB/c mice were i.p. injected with irinotecan (75 mg/kg) to induce mucositis and Km8154 (10^9 CFU) was orally administrated until the study endpoint. Seven days after the first dose, mice were euthanized, and intestinal samples were collected to asses clinical, histopathological and biochemical parame-

ters. Irinotecan antitumor activity was evaluated in a CT26 cells BALB/c mice tumour model, with tumour growth monitored daily. Tumour infiltrating cells were analysed by flow cytometry after euthanasia. **Results**: Irinotecan alters cellular tight junctions gene expression and promoted inflammation in intestinal epithelial cells, increasing claudin-1 and ccl20 expression, both attenuated by Km8154 ($p<0.01$). IL-8 expression was also reduced in cells co-treated with irinotecan and Km8154. Km8154 significantly protected against intestinal damage *in vivo*, improving villus/crypt ratios ($p=0.0001$), reducing intestinal shortening ($p=0.0049$), neutrophil infiltration ($p=0.027$), and oxidative stress ($p=0.0003$). Liver weight loss induced by irinotecan was also ameliorated with Km8154 treatment ($p<0.05$). Importantly, Km8154 did not interfere with irinotecan's antitumor efficacy, as indicated by similar percentage tumour growth (Irinotecan: 239±142%, Km: 255±70%), similar proportion of living cells and CD45+ tumour-infiltrating cells. **Conclusion**: The probiotic yeast Km8154 provides protective effects against irinotecan- induced intestinal mucositis without compromising chemotherapy efficacy.

422. 692. **SARM1 REGULATES ENERGY METABOLISM AND DIFFERENTIATION OF DISTINCT T LYMPHOCYTE SUBTYPES AND PROTECTS AGAINST THE DEVELOPMENT OF INTESTINAL INFLAMMATION**

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NAD+ is essential for T cell activation, requiring high levels of this electron acceptor. Recent research identified a NAD+-degrading domain in the SARM1 protein, which is expressed in CD4+ T lymphocytes. This led us to hypothesize that SARM1 might influence T cell quiescence, activation, differentiation, and memory formation by regulating NAD+ levels. In vitro experiments showed that SARM1 affects the differentiation of various lymphocyte populations. Specifically, in Th1 polarizing conditions, the absence of SARM1 resulted in an increased number of IFN+ cells. Conversely, SARM1 absence led to a rise in FOXP3+ cells during Treg and Th17 differentiation, with Th17 cells showing reduced IL-17 production. In vivo, Sarm1-/- mice displayed more activated T lymphocytes, as indicated by CD69 expression, but no change in T cell subpopulation numbers. When Rag1-/- mice were transferred with naive CD4+ T cells from Sarm1-/- donors, they developed more severe colitis compared to those receiving naive CD4+ T cells from wild-type mice. The Sarm1-/- group exhibited greater weight loss, splenomegaly, colon shortening, and intestinal barrier compromise, although the number of infiltrating T lymphocytes in the colon was unchanged after 30 days.Our findings suggest that SARM1 regulates the differentiation of Th1, Treg, and Th17 cells and modulates lymphocyte-mediated inflammation. Furthermore, reduced SARM1 expression was observed in circulating CD4+ T cells from colitis patients compared to healthy controls, supporting the notion that SARM1 is a key immunoregulatory component in CD4+ T lymphocyte biology.

423. 710. **EXPLORING THE IL-37/SIGIRR AXIS: A POTENTIAL PATHWAY IN ULCERATIVE COLITIS MANAGEMENT**

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Background: Ulcerative colitis (UC) is a chronic

inflammatory bowel disease marked by elevated levels of pro-inflammatory cytokines, leading to significant intestinal damage. IL-37 is an anti-inflammatory cytokine that mediates its effects through the formation of a tripartite complex with the receptors SIGIRR and IL-18R1. This pathway triggers the secretion of IL-10 and TGF-beta, key cytokines involved in controlling inflammation. Despite its potential importance, the role and involvement of IL-37 signaling axis in UC remain poorly understood. **Objective:** The main objective of this work was to evaluate the role of IL-37 and the SIGIRR receptor in patients with CU. **Methods:** We enrolled 12 patients with CU, all over 18 years of age, with a Mayo score (an indicator of disease activity) between 2 and 3; and six age and sex-matched healthy controls from the Hospital Clínico Universidad de Chile and Hospital San Juan de Dios in Chile. Colonic tissue biopsies were obtained during colonoscopy and RNA was isolated to evaluate the transcript levels of IL-37, SIGIRR, IL-18R1, IL-18, and IFN-y. The colonic tissue samples were then cultured and stimulated with recombinant IL-37 for 24 hours, after which proinflammatory cytokines in the supernatant were measured using cytometric bead analysis (CBA). **Results:** Colonic tissue from patients with CU exhibited significantly lower transcript levels of IL-37 and SIGIRR, alongside elevated levels of IL-18 and IFN-y, compared to healthy controls. Additionally, SIGIRR protein expression was reduced in inflamed colonic tissue relative to non-inflamed tissue. Notably, stimulation with recombinant IL-37 resulted in a trend towards reducing pro-inflammatory cytokines, including IL-18 and TNF-alpha, in inflamed colonic biopsies, indicating a potential therapeutic role for IL-37 in mitigating inflammation. **Conclusion**: These findings elucidate the role of the IL-37/SIGIRR axis in CU and suggest the potential of this pathway as a therapeutic target in this disease.

NEUROIMMUNOLOGY

424. 47. THE IMMUNOGENETIC ASPECT OF VITAMIN D METABOLISM IN MULTIPLE SCLEROSIS

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Background: The immunomodulatory effect of vitamin D (VitD) and its possible involvement in the susceptibility to multiple sclerosis (MS), an inflammatory T-cell mediated disease of the central nervous system, remain a hot scientific topic. **Aim:** Our aim was to analyze the effect of VitD on the innate (inflammation) and adaptive (Th17 pathway) immune system during MS in Tunisian patients. **Methods:** Samples of 318 individuals: 108 MS patients and 210 healthy controls were analyzed for determination of 25-(OH) VitD3 status. Circulating inflammatory cytokines (IL6, IL8, IL10, TNFα,IL12p70 and IL1β) were investigated using Cytometer Bead Array. A subpopulation (n=90; 45 MS patients-45 controls) benefited from a transcriptional study (VitD-related genes expression) and IL17A measurement. A cell culture step of PBMC +/-VitD stimulation was initiated with evaluation of the production of IL17A. **Results:** VitD was significantly lower in MS group, especially in female patients. Levels of TNFα was significantly higher in oligoclonal bands-positive MS patients. Circulating IL17A level was higher in patients compared to controls. The expression level of *VDR* gene was higher in MS group than in controls. The expression of *CYP27B1* gene was found to be significantly higher in controls. Regarding *IL23R* gene, its expression was significantly higher in MS patients compared to controls. Positive and significant correlations between the 3 genes expression were reported in the study population. The culture step revealed a decreased IL17A production in the supernatant with VitD. **Conclusion:** Our results support the association of VitD deficiency with MS. However, the vitamin level seems to not correlate with inflammatory cytokines nor with disability. The expression of the genes involved in VitD metabolism seems to be inter-correlated and in relation with IL23/Th17 pathway. The stimulation with VitD tends to inhibit differentiation towards the TH17 pathway. A project on miRNA profiling of the same cohort is in process.

425. 56. ROLE OF MENINGEAL LYMPHATIC VASCULATURE IN NEUROINFLAMMATION IN A MOUSE MODEL OF SIALIDOSIS

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Major advancements in neurobiology include the recent (re)discovery of a lymphatic vasculature within the meninges. Meningeal lymphatic vessels (mLV) are responsible for the drainage of immune cells, macromolecules, and fluid from the central nervous system (CNS) into the deep cervical lymph nodes (dCLNs). Impairment or malfunction of mLV may significantly impact on cerebrospinal fluid (CSF) clearance and exacerbate the buildup of protein aggregates. Yet, the contribution of defective mLV in neurodegenerative diseases is largely unexplored. The lysosomal storage diseases (LSDs), include a large group of pediatric neurodegenerative disorders caused by deficiency of lysosomal enzymes and associated with complex neurological phenotypes; however, the contribution of mLV to neurodegenerative LSDs, or the possible link between lysosomal function and mLV has not yet been explored. Here, we identify the sialidase neuraminidase 1 (Neu1) as the first lysosomal enzyme involved in the maintenance and preservation of mLV structure and function. In the mouse model of sialidosis, an LSD triggered by a deficiency of Neu1 (*Neu1*$^{-/-}$), we observed morphological defects of the mLV, characterized by excessive ectopic branching and increased area within the meninge; CSF accumulation in the cranium; and poor drainage of fluid into the dCLNs. Moreover, *Neu1*$^{-/-}$ mice display early infiltration of immune cells in their meninges prior to mLV morphological changes and are maintained throughout the course of the disease, contributing to the inflammation of the CNS. Our findings highlight the importance of lysosomal Neu1 in maintaining the mLV and therefore the proper CSF drainage and CNS homeostasis.

426. 258. NON-SPECIFIC ACTIVATION OF CD4+ T CELLS PROMOTES CORNEAL NEUROPATHY IN THE PROINFLAMMATORY CONTEXT OF DRY EYE

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Background: CD4+ T-cells promote tissue damage in dry eye (DED) but whether their ocular activation occurs in response to specific autoantigens or non-specific stimuli is unknown. **Objectives:** To evaluate the effect of restricted CD4+ T cell-receptor (TCR) specificity on the development of DED to indirectly assess the contribution of potentially autoreactive CD4+ T cells to DED pathogenesis. **Methods:** DED progression was assessed after extraorbital lacrimal gland excision in wild-type (WT, full TCR repertoire), OT-II (reduced TCR repertoire), Rag1KO/OT-II (single TCR specificity), and Rag1KO mice (no T cells) mice. Corneal epitheliopathy was measured by fluorescein-dextran uptake, corneal nerve function by mechanical and capsaicin sensitivity, and corneal nerve density by confocal microscopy. Also, CD4+ T cells were adoptively transferred into Rag1KO recipients. All statistical analyses were performed using Student's t test. **Results:** Corneal epithelial staining increased comparably in all groups of DED mice ($p>0.05$). By contrast, corneal mechanical sensitivity decreased on days 5 and 10 in WT (-10%, -20%), OT-II (-4%, -18%), and Rag1KO/OT-II (-12%, -17%) but not in Rag1KO mice (-1%, -1%). Consistently, capsaicin sensitivity increased in WT (+14%, +22%), OT-II (+13%, +17%), and Rag1KO/OT-II (+30%, +26%) but not in Rag1KO mice (-2%, +5%). Corneal nerve density decreased only in WT DED mice. Corneal mechanosensitivity decreased comparably in Rag1KO mice after 3 weeks of adoptive CD4+ T cell transfer from WT (-33%), OT-II (-31%), and Rag1KO/OT-II (-33%) DED mice. **Conclusion:** Corneal epitheliopathy in DED does not depend on the presence or specificity of CD4+ T cells whereas corneal nerve dysfunction develops only in the presence of these cells but irrespective of their specificity. However, DED-associated morphological changes in corneal nerves require a full CD4+ T cell repertoire. Thus, both antigen-specific and bystander CD4+ T cell activation contribute to corneal neuropathy in DED

427. 301. IDENTIFICATION OF SEROTONYLATION EVENTS IN A MURINE MODEL OF REACTIVE ARTHRITIS

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Background: Reactive arthritis (ReA) prone mice have higher basal serotonin (5-HT) serum levels, and selective serotonin reuptake inhibitors (SSRIs) that reduce it have an anti-inflammatory effect. Combining TG2 inhibitors with SSRI significantly reduces the severity of ReA in a murine model, potentially by inhibiting pos-traductional modifications like serotonylation. Nevertheless, the precise mechanism remains unclear. **Objectives:** To evaluate the impact of 5-HT and serotonylation on ReA development. **Methods:** TNF receptor 1 knockout (TNFR1 KO) mice, prone to ReA after *Yersinia enterocolitica* (Ye) infection, were used. Mice were orally infected with Ye O:3, treated with the 5-HT precursor (5-HTP) to elevate serum 5-HT, and ReA clinical scores were recorded in surviving mice. On day 21 post-infection (dpi), mice were euthanized, and flow cytometry (FC) was performed on regional lymph nodes (RLN) to detect dendritic cells (DCs), macrophages, neutrophils and T cell infiltrates. Additionally, splenocytes from TNFR1 KO mice with ReA untreated or treated with fluoxetine (SSRI) and cysteamine (TG2 inhibitor) were assessed at different dpi for serotonylation using click reactions and FC. Statistical analyses were conducted using one-way ANOVA and student t-tests. **Results:** Increased 5-HT exacerbates ReA severity (p=0,0034) and increases neutrophil infiltrates in RLN (p=0,0465) without changes in other cell populations. Additionally, spleens from ReA mice had a higher number of serotonylated splenocytes (p=0,042) and mean fluorescence intensity (MFI, p=0,0011). Interestingly, serotonylated splenocytes increased at 5 dpi (p=0,0005) without an MFI increment. Besides, inhibitor treatments did not reduce the number of labeled cells but tended to lower MFI per cell. **Conclusion:** In conclusion, Ye infection increases serotonylation levels, which may contribute more significantly to ReA than the number of serotonylated cells. Besides, elevated 5-HT aggravates ReA severity, leading to an increased neutrophil infiltration in RLN. Our findings suggest that the increased serotonylation events may contribute to sustain inflammatory conditions.

428. 391. KETAMINE REDUCES PERIPHERAL AND BRAIN INFLAMMATION BY PROMOTING REGULATORY IMMUNE CELLS, ALLEVIATING LPS-INDUCED DEPRESSIVE-LIKE BEHAVIOR IN MICE

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Background. Ketamine, a NMDA receptor antagonist, could act as rapid antidepressant, but its therapeutic action cannot be explained by the only receptor antagonism in the CNS. **Objective.** We aimed to determine if ketamine's action on the immune system contributes to its antidepressant effect in an LPS-induced depression model. **Methods.** Adult male C57BL/6J mice were intraperitoneal injected with 1 mg/kg LPS, and 24h later, treated with 10-20 mg/kg of ketamine. Anhedonia and hopelessness were measured by the sucrose preference test (SPT) and the tail suspension test (TST). After 72h, 100µl of blood was obtained from the submaxillary vein, and mice were euthanized. Cardiac perfusion was performed, and the spleen and brain were removed for immunological analysis by flow cytometry. ANOVA or Kruskal-Wallis test was performed. **Results.** LPS-treated animals consumed less sucrose ($p<0.001$) and were immobile longer than controls ($p<0.001$). Ketamine reduced immobility time in the TST ($p<0.05$) and increased sucrose preference in the SPT compared to LPS-treated mice. In the peripheral circulation, LPS increased the proportion of granulocytic cells compared to the control ($p<0.001$). Ketamine reduced the percentage of granulocytic cells ($p<0.05$) and increased the $CD11b^{+}Ly6C^{neg}$ regulatory monocytes ($p<0.05$) compared to LPS-treated mice. In the brain, LPS-treated mice showed a greater percentage of activated microglia with high CD11b levels and a higher proportion of infiltrating monocytes compared to controls ($p<0.05$). Ketamine decreased the percentage of activated microglia ($p<0.05$) and reduced monocyte infiltra-

tion in depressed mice ($p<0.01$). In the spleen, LPS-treated mice exhibited a reduced CD4/CD8 ratio ($p<0.001$) and an increased percentage of $CD3^{+}CD4^{neg}CD8^{neg}$ cells ($p<0.0001$), along with a higher proportion of macrophages expressing lower CD206 levels ($p<0.0001$) compared to controls. Ketamine promoted CD206 expression ($p<0.05$) and significantly increased $CD4^{+}CD69^{+}$-TIM-3^{+} regulatory lymphocytes in depressed mice ($p<0.01$). **Conclusion.** These results highlight ketamine as a potential treatment for inflammation-related depression.

429. 424. **THE EFFECT OF LEAD (PB) ON THE IMMUNE RESPONSE AND WORKING MEMORY OF CHILDREN AND ADULTS FROM HUAUTLA, MORELOS**

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In the Sierra de Huautla Biosphere Reserve in Morelos, México, significant mining waste containing heavy metals like Pb has accumulated. Pb can directly impact the central nervous system by entering the brain, affecting neuronal communication, and modulating the immune system (IS), including the proliferation of IS cells and cytokine expression. These effects may also influence brain regions associated with working memory. However, the interaction between these systems in human populations remains poorly understood. This study aims to assess the impact of Pb exposure on cytokine expression and the phenotypic profile of IS cells, alongside evaluating working memory performance in children and adults living in Huautla, Morelos. Pb concentrations were measured in hair samples using voltammetry. Working memory was assessed with the Neuropsychological Battery of Executive Functions Frontal Lobes, and serum cytokines were quantified through a multiplex assay. Peripheral blood mononuclear cells were isolated, cryopreserved, and later stimulated for flow cytometry analysis of T lymphocyte subgroups. Preliminary findings revealed significantly higher Pb concentrations in adult males (n=42) compared to females (n=78; U-Mann Whitney), and higher working memory scores in females (n=15) than in males (n=10; Student's t-test). These sex differences may result from cultural and physiological factors, such as hormonal differences. Notably, Pearson's correlation indicated a significant negative relation between Pb levels (below 2mg/kg) in hair samples and pro-inflammatory cytokines IL-17A, IL-23, IL-33, IFN-alpha, and IFN-gamma in both sexes. These preliminary results suggest that Pb exposure is associated with altered cytokine expression, which may relate to working memory performance, and the ongoing analyses will further explore these immune cell populations' effector and activator phenotypes.

430. 479. **SYSTEMIC IL-6 INCREASE FOLLOWING HUMAN METAPNEUMOVIRUS INFECTION PROMOTES BLOOD-BRAIN BARRIER PERMEABILITY AND NEUROINFLAMMATION IN MICE**

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Background: The human metapneumovirus (hMPV) is a major viral agent known for causing acute lower respiratory tract infections, affecting mainly the pediatric population. Even though the most commonly severe symptoms of the infection are bronchiolitis and pneumonia, some of these patients develop neurological manifestations, such as encephalitis. Therefore, this work seeks to evaluate possible brain alterations after hMPV infection. **Methods:** To assess the effects of the infection with hMPV on the brain, BALB/c mice were challenged intranasally with non-infectious control (mock), UV-inactivated hMPV (UV-hMPV), or a clinical hMPV isolate. 1- 3- and 7-days post-infection (dpi), brain and serum samples were collected. **Results:** the brains of the hMPV-infected mice showed a significantly increased relative expression of cytokines (IL-6, IL-1□, and TNF-a), glial markers (GFAP), and cell junction complexes (claudin-5, ICAM-1, and VCAM-1), compared with uninfected mice. Since the cell junction complexes are key components of the blood-brain barrier (BBB), the permeability of this barrier was evaluated by an Evans Blue assays. A significant increase of BBB permeability was observed at 3 dpi in hMPV-infected mice.

Despite the inability to detect viral load in the brains of the infected mice, a systemic increase of pro-inflammatory cytokines such as IL-6 and IL-1 was detected in the blood of infected mice. Therefore, to evaluate if the systemic secretion of IL-6 could promote the increased permeability of the BBB, mice were treated with an IL-6 neutralizing antibody the infection with hMPV. Mice treated with anti-IL-6 before the infection with hMPV demonstrated a decrease in the permeability of the BBB. **Conclusion:** Our results suggest that infection with hMPV causes the systemic increase of pro-inflammatory cytokine IL-6, which promotes the increased permeability of the BBB and the neuroinflammation in the brain.

431. 555. CD4+ T CELLS DRIVE CORNEAL NERVE DAMAGE BUT ARE DISPENSABLE FOR CORNEAL EPITHELIOPATHY DEVELOPMENT IN THE CONTEXT OF DRY EYE

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Background: Whether corneal neuropathy in dry eye disease (DED) is a consequence of corneal epithelial damage or caused by pathogenic CD4+ T cells is controversial. We tested the hypothesis that immune-driven corneal neuropathy in DED occurs independently of corneal epitheliopathy. **Objectives:** to explore the relative contribution of the adaptive immune response, and more specifically of CD4+ T cells, to corneal epithelial and nerve damage in a murine model of DED. **Methods:** DED was induced by extraorbital lacrimal gland excision in wild-type (WT) and T cell- deficient (*Rag1*KO) mice. Corneal epithelial integrity and nerve function were measured by dextran-FITC uptake and mechanosensitivity, respectively. Corneal nerve morphology was evaluated by whole-mount confocal microscopy while trigeminal ganglion gene expression was analyzed by RNA-seq. Also, CD4+ T cells from DED or sham-surgery WT mice were adoptively transferred to *Rag-1*KO mice. To determine differences, Student's t-test was used. **Results:** Compared to sham mice, WT and RAG1KO DED mice developed a similar increase in corneal fluorescein uptake (d10:290%vs266%,p=0.93). By contrast, corneal mechanosensitivity progressively dropped only in DED WT mice (d10:-21vs+1%,p=0.01) and there was a larger DED- induced decrease in subapical (-54vs-17%,p<0.001), mid-epithelial (-48vs-19%,p=0.02), and subbasal (-36vs-8%,p=0.01) nerve density in WT than in RAG1KO mice. RNA-seq of trigeminal ganglia from WT and RAG1KO mice identified 172 and 7 differentially expressed genes induced by DED, respectively. Further enrichment analysis found neurodegeneration- and neuropathic pain-associated pathways only in DED WT mice. DED WT CD4+ T cell transfer to naïve RAG1KO mice did not affect corneal epithelial barrier function but significantly decreased corneal mechanosensitivity (-21%,p<0.01) and corneal nerve density (p<0.01) at the subapical (-40%), mid-epithelial (-42%), and subbasal (-32%) levels. **Conclusion:** Our results indicate that in DED, CD4+ T cells drive corneal neurodegeneration but are not required for corneal epithelial damage to develop. This finding holds profound implications in DED therapy.

432. 556. INTERFERON-GAMMA INDUCES TOLEROGENIC PHENOTYPE AND ACTIVITY IN MONOCYTE-DERIVED DENDRITIC CELLS FROM PATIENTS WITH MULTIPLE SCLEROSIS AND HEALTHY DONORS

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Our previous results have shown that low doses of interferon-gamma (IFN-g) induce differentiation of murine bone marrow-derived dendritic cells (BMDCs) with tolerogenic phenotype and function and with therapeutic activity in experimental autoimmune encephalomyelitis (EAE), a pre-clinical model of MS. In this study, we determined the impact of IFN-g on the differentiation and func-

tion of monocyte-derived dendritic cells (moDC) from patients with multiple sclerosis and healthy donors (HD). Monocytes were isolated from peripheral blood of untreated MS patients (n=11) or HD (n=9) by CD14-specific immunobeads. moDC were differentiated using GM-CSF and IL-4 (1000 U/ml) in the absence (UN-moDC) or presence of increasing concentrations (1 to 1000 ng/ml) of IFN-g (IFN-g-moDC). Lipopolysacharide (LPS, 1 mg/ml) was added during the last 24 h to obtain mature moDC (m-moDC) and to evaluate functional stability of IFN-g- moDC (LPS-IFN-g-moDC). Cell viability, DC yield, and tolerogenic phenotype were determined by flow cytometry. The tolerogenic function was evaluated in a mixed lymphocyte reaction (MLR) assay by co-culturing IFN-g-moDC or LPS-IFN-g-moDC with Cell Trace Violet (CTV) labeled allogeneic peripheral blood mononuclear cells from HD. $CD4^+$ and $CD8^+$ T cell proliferation and activation were determined by flow cytometry. We found that IFN-g-moDC from MS patients and HD exhibited a tolerogenic phenotype characterized by significantly lower levels of CD80 than m-moDC and significantly higher levels of PD-L1 than UN-moDC. Additionally, IFN-g-moDC from HD had significantly lower levels of CD86 and CD83 than m- moDC. Remarkably, IFN-g-moDC and LPS-IFN-γ-moDC from MS patients and HD significantly inhibited $CD4^+$ and $CD8^+$ T cell proliferation and activation. LPS-IFN-g-moDC from MS patients significantly inhibited $CD4^+$ T cell proliferation and activation. Our results demonstrate that IFN- g induces differentiation of moDC from MS patients and HD with tolerogenic phenotype and function.

433. 559. **P2X7 RECEPTOR ANTAGONISM RELIEVES DEPRESSIVE-LIKE BEHAVIOR IN MICE FOLLOWING CHRONIC ULCERATIVE COLITIS**

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Background: Inflammatory bowel disease (IBD) is a multifactorial disorder that is characterized by chronic intestinal inflammation. Patients with IBD commonly have psychiatric disorders, including depression. Adenosine triphosphate (ATP) is a potent alarmin released by cellular stress/inflammation that modulates immune responses. ATP can activate the P2X7 receptor, widely expressed in immune cells and the central and enteric nervous systems. This receptor may contribute to the pathology of IBD. Here, we investigated the role of the P2X7 receptor in colitis-induced behavioral changes in mice. **Objectives:** We sought to investigate the role of the P2X7 receptor in colitis-induced behavioral and cellular changes in sex-specific mice. **Methods**: Chronic colitis was induced in C57Bl/6 mice (8-10 weeks) (male and female) with two cycles of dextran sodium sulfate (DSS) 2% (w/v) diluted in water for 7 days and 10 days drinking water between cycles. Mice received the specific P2X7 receptor antagonist Brilliant Blue G (BBG) (45 mg/kg) intraperitoneally. Object recognition and tail suspension test (ORT and TST) and molecular and cellular analyses (western blotting, Real-time PCR, flow cytometry, and histology) were performed. **Results**: DSS-treated animals showed increased P2X7 receptor expression in the gut. DSS-BBG animals showed a smaller and slower weight loss than the control group (DSS-vehicle). Colitis also increases glial fibrillary acidic protein (GFAP) and caspase-3 in the gut. BBG treatment reversed these effects. BBG also decreased the populations of Tregs and Th17 cells in male mice with colitis. However, in female mice, BBG treatment did not prevent the increase in these populations. TST showed colitis-induced depression-like behavior in male and female mice, which was prevented by BBG treatment. **Conclusion**: Our results show that P2X7 receptor inhibition significantly reduces colitis-induced inflammation and alleviates depression-like behavior, with slightly sex-specific variations, highlighting the key role of this receptor in modulating the gut-brain axis in inflammatory bowel disease.

434. 582. **THE ROLE SUBSTANCE P-INDUCED MAST CELL ACTIVATION IN LIVER FIBROSIS IN THE METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD)**

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Introduction: MASLD progression is associated to psychosocial stress and a high density of liver mast cell (MCs). Despite hepatic stellate cells (HSCs) activation by MC mediators has a role in liver fibrosis, the neuroimmune activation of MC by stress-related neuropeptide Substance P(SP) remains unexplored. **Objective:** To evaluate psychosocial stress in MASLD patients and the role of SP-dependent MC activation in HSCs transdifferentiation (HSCT). **Methods:** Transversal study in 13 MASLD patients diagnosed by liver biopsy, recruited from Gastroenterology Unit- HCUCH. Psychological stress was assessed by self-report questionnaires (PSS-14, HADS, and SF-36). Liver damage, by blood test and imaging. Study approved by HCUCH Ethics Committee. *In vitro*, Human mast cell line (HMC-1), stimulated with SP and 48/80 compound (30min), and HSCs cell line (LX-2), subsequently stimulated by 24h with supernatants of SP-MC activated, were assessed for alfa- smooth muscle actine (α-SMA) and GADPH expression by Immunoblot. A direct tryptase stimulation of HSCs by 24h was performed and α-SMA immunostaining was assess by IFI. Statistical analyses: t-Test, chi-square test for clinical study; and ANOVA-test for *in vitro* studies, using GraphPad-Prisma®. Significance $p<0.05$. **Results:** Moderate-stress in 53.8% of participants, and a higher stress score in patients with moderate-severe steatosis (29.0±9.51) vs mild-steatosis (18.20±6.94; p=0.047), was observed. Patients with significant-fibrosis reported poorer mental Quality of Life (QoL) (42.40±10.40) vs without-fibrosis (53.60±9.25; p=0.022). Despite not statistically differences in fold change of α-SMA/GADPH level expression among MC stimulated conditions (unstimulated MC,0.394±0.08;48/80-MC,0.256±0.130;SP-MC,0.274±0.120;p=0.622), morphological changes associated to HSCT were observed after tryptase stimulation. **Conclusion:** Our results suggest that negative psychosocial factors have a pathogenic role in liver damage progression. Our *in vitro* study indicates that MC tryptase can induce HSCT; however short-term effect of SP-MC activation fail to achieve in HSCs activation, suggesting long term MC stimulation need to be explored to evaluate SP-MC impact in HSCT. Funding OAIC13022.

435. 618. INTERFERON-GAMMA INDUCES TOLEROGENIC DENDRITIC CELLS WITH THERAPEUTIC ACTIVITY IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Our previous results have shown that interferon-gamma (IFN-g) suppresses experimental autoimmune encephalomyelitis (EAE), a pre-clinical model of MS, by inducing splenic $CD11b^+$ myeloid cells with tolerogenic and therapeutic activities. In this study, we assessed the *in vitro* effect of IFN- g on the differentiation, tolerogenic phenotype, and therapeutic activity of murine bone marrow- derived dendritic cells (BMDCs). BMDCs precursors from mice were differentiated into dendritic cells (DC) with GM-CSF (20 ng/ml) for 7 days in the absence (iDC) or presence of IFN-g (IFN-g-DC). Different concentrations of IFN-g (5 to 50 ng/ml) were added starting from day 0, 2 or 4 of differentiation. Lipopolysacharide (LPS, 100 ng/ml) was added during the last 24 h to obtain mature DC (mDC) and to evaluate phenotypic and functional stability of IFN-g- DC (LPS-IFN-g-DC). Cell viability, DC yield, phenotypic profile, and expression of indoleamine 2,3-dioxygenase 1 (IDO-1) and Aryl hydrocarbon receptor (AhR) were determined by flow cytometry. The tolerogenic function was evaluated in a mixed lymphocyte reaction (MLR) assay. EAE mice were i.v. transferred with IFN-g-DC or LPS-IFN-g-DC pulsed with myelin oligodendrocyte glycoprotein (MOG) peptide at the peak of disease and clinical progression was monitored daily. We found that DC differentiated in the presence of IFN-g exhibited a tolerogenic phenotype characterized by significantly lower levels of CD80, CD86, and MHC-II, and significantly higher levels of PD-L1 than mDC. LPS-IFN-g-DC showed a stable phenotype. Induction of tolerogenic IFN-g-DC was associated with increased expression of IDO-1 and

AhR. Tolerogenic IFN-g-DC and LPS-IFN-g-DC inhibited antigen-specific CD4+ T cell proliferation and activation. Remarkably, adoptive transfer of either IFN-g-DC or LPS-IFN-g- DC ameliorated clinical symptoms of EAE. Therefore, low doses of IFN-g induce differentiation of murine DC with tolerogenic phenotype and function and with therapeutic activity in EAE.

436. 619. **THERAPEUTIC ROLE OF INTERFERON-GAMMA IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IS MEDIATED THROUGH A TOLEROGENIC SUBSET OF SPLENIC CD11B+ MYELOID CELLS**

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Cumulative evidence has established that interferon-gamma (IFN-g) has both pathogenic and protective roles in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). However, the mechanisms whereby IFN-g may promote neuroprotection in EAE are poorly understood. In this study, we addressed the impact of IFN- g on peripheral and CNS infiltrating CD4+ T cell subpopulations in EAE. The frequency of regulatory T (Treg) cells in spinal cords from chronic EAE mice treated with IFN-g was significantly increased with no effect on Th1 and Th17 cells. Consistently, depletion of FOXP3- expressing cells blocked the protective effects of IFN-g, indicating that the therapeutic effect of IFN-g depends on the presence of Treg cells. However, IFN-g did not trigger direct *in vitro* differentiation of Treg cells. *In vivo* administration of blocking antibodies against either interleukin (IL)-10, transforming growth factor (TGF)-β or program death (PD)-1, revealed that the protective effects of IFN-g in EAE were also dependent on TGF-β and PD-1, but not on IL- 10, suggesting that IFN-g might have an indirect role on Treg cells acting through antigen- presenting cells. Indeed, IFN-g treatment increased the frequency of a subset of splenic CD11b+ myeloid cells expressing TGF-β-Latency Associated Peptide (LAP) and program death ligand 1 (PD-L1) in a signal transducer and activator of transcription (STAT)-1-dependent manner. Furthermore, splenic CD11b+ cells from EAE mice preconditioned *in vitro* with IFN-g and myelin oligodendrocyte glycoprotein (MOG) peptide exhibited a tolerogenic phenotype with the capability to induce conversion of naïve CD4+ T cells into TGF-β-secreting Treg cells. Remarkably, adoptive transfer of splenic CD11b+ cells from IFN-γ-treated EAE mice into untreated recipient mice ameliorated clinical symptoms of EAE and limited central nervous system infiltration of mononuclear cells and effector helper T cells. Therefore, IFN-g promotes beneficial effects in EAE by endowing splenic CD11b+ myeloid cells with tolerogenic and therapeutic activities.

437. 642. **NEUROIMMUNOLOGICAL CHARACTERIZATION OF ALTERATIONS INDUCED BY HUMAN RESPIRATORY SYNCYTIAL INFECTION**

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Respiratory viruses are the leading cause of acute lower tract respiratory infection, mainly in infants, elderly, and immunocompromised individuals, causing high morbidity and mortality rates. The most important of these viruses is the respiratory syncytial virus (hRSV), which can cause severe clinical pathologies, including bronchiolitis and pneumonia. However, increasing evidence shows this virus's ability to cause neurological alterations, such as seizures, encephalitis, and encephalopathy. Reports have been shown to detect hRSV RNA and pro-inflammatory molecules in cerebrospinal fluid from patients with neurological signs, supporting the notion of neuroinvasion and neuroinflammation caused by hRSV. Studies in animals have shown the detection of viral RNA and proteins in the brains of infected mice. Besides, hRSV can cause long-term sequelae such as learning and behavioral impairment months after the viral clearance. Additionally, the viral infection alters the blood-brain barrier

permeability, allowing the immune cells to infiltrate and increase pro-inflammatory molecules. Moreover, hRSV can infect astrocytes, microglia, neurons, and endothelial cells. Since previous data has shown the detection of viral RNA and proteins in the brains of infected mice, we want to explore the neuroimmunological alterations induced by hRSV in mice and *in vitro* models. Using four to six-week female mice, we perform an hRSV infection kinetics at 1-, 3-, 7-, 9, and 60-days post-infection to evaluate the tight junctions' expression, glutamate receptor NMDA(R) and neurotrophins by qRT-PCR. Moreover, hRSV can infect a primary culture of mice endothelial vascular cells and induce the secretion of cytokines. To evaluate the hRSV infection on neurons, we use the HT22 cell line to perform infection kinetics at 24, 48, and 72 hrs. We observed that hRSV infection alters the tight junctions' expression in the brains of infected mice. Also, the NMDAR subunits are increased at 60 days post-infection. Likewise, BDNF and NGF, as well as their receptors, also are increased at 60 days post-infection. Taken together, our work provides new insights into the effects of hRSV on the central nervous system and calls for attention to assess further this virus's impact on the brain and its function in humans. **Funding:** This work was supported by ANID/FONDECYT grants #11221280; #1190830, the Millennium Institute on Immunology and Immunotherapy ACE 210015, ICN09_016 / ICN 2021_045; former P09/016-F. Respiratory viruses, particularly the respiratory syncytial virus (hRSV), are major causes of acute lower respiratory infections, especially in infants, the elderly, and immunocompromised individuals, leading to significant morbidity and mortality. While hRSV is primarily associated with severe respiratory conditions like bronchiolitis and pneumonia, growing evidence indicates its capacity to induce neurological complications, including seizures, encephalitis, and encephalopathy. Detection of hRSV RNA and pro-inflammatory molecules in the cerebrospinal fluid of patients with neurological symptoms supports the idea of hRSV-induced neuroinvasion and neuroinflammation. Animal studies have further corroborated these findings, revealing the presence of viral RNA and proteins in the brains of infected mice. hRSV infection can result in long-term neurological sequelae, such as learning and behavioral impairments, persisting months after viral clearance. This infection also disrupts the blood-brain barrier, facilitating immune cell infiltration and elevating pro-inflammatory molecules. Additionally, hRSV can infect various brain cells, including astrocytes, microglia, neurons, and endothelial cells. Our research aims to explore the neuroimmunological alterations induced by hRSV using both in vivo and in vitro models. In four- to six-week-old female mice, we conducted hRSV infection kinetics at 1, 3-, 7-, 9-, and 60-days post-infection, assessing tight junction expression, NMDA receptor subunits, and neurotrophins via qRT-PCR. In parallel, hRSV's impact on endothelial vascular cells and the HT22 neuronal cell line was studied, focusing on cytokine secretion and infection kinetics. We found that hRSV infection altered tight junction expression in the brain and increased NMDAR subunits, BDNF, and NGF levels at 60 days post-infection. Our findings provide novel insights into hRSV's effects on the central nervous system, underscoring the need for further investigation into its impact on human brain function.

438. 722. LEPTIN SIGNALING IN MICROGLIA: A PIVOTAL ROLE IN OBESITY-ASSOCIATED NEUROINFLAMMATION AND METABOLIC DYSFUNCTION

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Obesity, characterized by excessive adipose tissue accumulation due to chronic energy imbalance, leads to elevated levels of circulating and hypothalamic saturated fatty acids, such as palmitate. Chronic exposure to high palmitate levels triggers pathological events including metabolic disorders, chronic inflammation, neurodegeneration, and insulin resistance. Obesity is also linked to leptin resistance, a crucial adipokine involved in feeding behavior regulation. In the CNS, microglia detect increased leptin and palmitate, initiating an inflammatory response in the hypothalamus, key for appetite and energy metabolism regulation. However, how leptin influences microglial activation during obesity remains unclear. This study aims to determine how hyperleptinemia modulates microglia in obesity. We used tamoxifen-induced CX3CR1$^{CreER/+}$ (Obr$^{+/+}$) animals as controls and Obr $^{fl/fl}$ CX3CR1$^{CreER/+}$ (Obr$^{-/-}$) animals with conditional deletion of the leptin receptor (ObR) in microglial cells. After twenty weeks of diet, we observed a paradoxical phenotype in Obr$^{-/-}$ animals, characterized by reduced food intake but increased body weight, along with glucose intolerance and insulin resistance. Furthermore,

high-fat diet feeding increased IL-6 and TNF-α expression in the hypothalamus of $Obr^{-/-}$ animals. To investigate the metabolic mechanism, primary microglia were cultured and treated with leptin and palmitate. Palmitate, but not leptin, altered mitochondrial morphology, reducing branching. This morphological change increased glycolysis and cellular respiration in $Obr^{-/-}$ microglia. Additionally, deletion of ObR resulted in the loss of palmitate-induced IL-6 secretion, suggesting that ObR is crucial for metabolic regulation. In conclusion, leptin receptors in microglia play a fundamental role in maintaining energy homeostasis, and disruptions in leptin signaling may activate compensatory mechanisms contributing to obesity and metabolic dysfunction.

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439. 002. CANCER NEUTROPHIL ENCYCLOPEDIA: A DEEP DIVE INTO ANTIGEN-PRESENTING WARRIORS

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Neutrophils, the most efficient defenders against pathogens, are essential for tumor microenvironment balance and homeostasis. However, given their plasticity and short half-life which made them too fragile to be profiled, it poses complex challenges regarding how neutrophils are imprinted and adapt specific fates across cancers. Here we designed a one-two-punch sorting strategy, generated the neutrophil atlas from 225 samples of 144 patients from 17 cancer types, and further developed a computational pipeline to recover both shared and specific transcriptional programs. Unexpectedly, neutrophils harbored extraordinary complexity composed of 10 cell states and showed sharp tissue or phenotypic specialty. We observed and verified that cancer neutrophils are dramatically arranged along tumor-specific terminal differentiation paths such as inflammation, angiogenesis and antigen- presenting. In particular, the antigen-presenting program was associated with better patient outcomes in the majority of cancers. Such a program can be evoked by leucine metabolism and is dependent on mitochondrial remodeling, acetyl-CoA generation, and preferable epigenetic histone H3K27ac modification. Functionally, antigen-presenting neutrophils invoked expanded T cell response and neoantigen-specific reactiveness. We finally designed the antigen-presenting neutrophil immunotherapy (adoptive transferring and leucine diet) which fine-tunes the microenvironment balance and fuels anti-PD-1 immunotherapy. In summary, these data not only lay the groundwork for future neutrophil research, and open the black box of neutrophil state divergence across cancers, but also unravel minimally invasive therapeutic opportunities including adoptive transferring antigen- presenting neutrophils.

440. 016. USING HELMINTH-DERIVED MOLECULES TO IMPROVE CONVENTIONAL CHEMOTHERAPY FOR EXPERIMENTAL COLORECTAL CANCER

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Background: Colorectal cancer (CRC) is among the deadliest neoplasias worldwide. In Mexico, it just reached number 1 for cancer-associated deaths. Notwithstanding its low efficiency, 5-Fluorouracil (5-FU) is a chemotherapeutic agent utilized to treat CRC. The factors that give rise to CRC include chronic inflammation; however, helminth infections have been reported to inhibit inflammatory responses. Despite this, the role of helminths in cancer development has still not been thoroughly explored. Objectives: To determine the effects of excreted/secreted products of the helminth Taenia crassiceps (TcES) as an adjuvant for 5-FU in a murine model of colitis-associated colon cancer (CAC). Methods Eight-to ten-week-old female BALB/c mice were induced to CAC with azoxymethane (12.5mg/kg) and dextran sulfate sodium (DSS) (2%) in drinking water. On day 54 after CAC-induction, mice were inoculated with 200µg of TcES 3 times per week, and on day 60, mice received 5-FU (30mg/kg) together with TcES or single 5-FU or saline and were euthanized on day 80. Results. Treatment with TcES plus 5-FU reduced IL-1β, TNF-α, and IL-17 production, whereas it inhibited colon tumorigenesis by downregulating genes related to drug resistance and Ki-67 and Cyclin D1. Also, TcES plus 5-FU increased the recruitment of NK cells, which secreted more granzyme B. Conclusion: Our study demonstrates a remarkable effect of TcES on suppressing ongoing colorectal cancer by downregulating proinflammatory and

pro-tumorigenic signaling pathways, improving the 5-FU effect. Funding Support: Program for Research Projects and Technological Innovation (PAPIIT)-UNAM, grant number (IN-212722).

441. 036. **3D CO-CULTURE SYSTEM FOR THE DEVELOPMENT OF A CHEMORESISTANCE PREDICTION PLATFORM IN B-ALL**

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The expansion of malignant lymphoid precursors within the bone marrow results in B-cell acute lymphoblastic leukemia (B-ALL), the main population affected are children. In the past decade there have been remarkable advances in the definition of the molecular abnormalities involved in leukaemogenesis and drug resistance, due to ability of hosting and protecting leukemia initiating cells in the leukemic niches. The objective of the investigation is to evaluate the tumor microenvironmental to establish a 3D co-culture system treated with chemotherapeutics frequently used in the treatment of B-ALL. For this purpose, generic organoids were formed from stromal mouse cells OP9 and lymphocyte-like Nalm6 cells, they were treated with concentrations 50,25,12.2,6.25,3.12 ng/ml of vincristine, also the cell line Reh was evaluated with the same conditions. Samples were acquired in the cytometer to detect $CD45^+$ and 7AAD to determine the treatment effectiveness on the cells that migrate in the interior of the organoid, and the exterior cells. The results showed there are no significant difference between the different concentrations. Both cell lines were evaluated at different concentrations of daunorubicin, and both results showed significant difference between concentrations with these chemotherapeutics and interior and exterior cells. In conclusion the Nalm 6 cell line and Reh showed to have chemoresistance to vincristine, even at concentrations at which cell samples isolated from patients showed sensitivity, which let us establish these cell lines as a chemoresistant controls, however both cell lines could be established as chemosensitive controls to daunorubicin. This methodology will let us evaluate new drugs, the resistance or sensitivity of cell samples isolated from patients to generate a better diagnosis.

442. 039. **PROSPECTIVE MULTICENTRIC VALIDATION OF THE CONSENSUS IMMUNOSCORE IN STAGE I-III COLON CANCER: A PROGNOSTIC AND CLINICAL IMPACT STUDY FOR PATIENT MANAGEMENT**

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Background: A retrospective multi-institutional international validation study has demonstrated the prognostic value of the Immunoscore (IS) test in patients with colon cancer. **Objectives:** This study aimed to prospectively validate the prognostic impact of the IS in routine care for patients with UICC-TNM stage I-III colon cancer (NCT01688232, NCT02274753). **Methods:** Multicenter cohorts, including a test cohort (n=206) and a validation cohort (n=235), were investigated. Densities of CD3+ and CD8+ T cells in the tumor and its invasive margin were assessed us-

ing immunohistochemistry and digital pathology to determine the IS. The primary endpoint was disease-free survival (DFS). Stratified multivariable Cox models were used to assess the associations between IS and outcomes. **Results:** IS-Low, IS-Intermediate, and IS-High were observed in 19.0%, 54.2%, and 26.8% of patients, respectively. In the test cohort, IS-High was significantly associated with a lower risk of relapse or death compared to IS-Low (HR=2.93, 95% CI 1.45-5.93; P=0.007). These findings were confirmed in the validation cohort (P=0.007). In the subgroup of microsatellite-stable patients, a significant association between IS and time to relapse (TTR) was found (P=0.017). In multivariable analysis across all patients, IS (Low vs High) was significantly associated with DFS and TTR (P=0.0013 and P<0.0001, respectively), when adjusted for sex, age, T/N stage, and tumor sidedness. In clinically high- and low-risk stage III patients, IS stratified patients with distinct clinical outcomes (5-year recurrence-free rates from 39.3% to 95.6%; P<0.00001). Among stage II patients, those at clinically high-risk with IS-Intermediate presented with similar outcomes, and those with IS-High with better outcomes compared to clinically low-risk patients. **Conclusion:** The IS provides a reliable estimate of the risk of recurrence in patients with stage I-III colon cancer, supporting its implementation in routine clinical practice to optimize medical surveillance and indications for adjuvant chemotherapy.

443. 052. **MIF AS AN ENHANCER OF MALIGNANCY IN COLITIS-ASSOCIATED COLORECTAL CANCER**

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Colorectal cancer (CRC) is the second leading cause of death by cancer worldwide. Inflammatory bowel diseases (IBD) increase up to 20 % of the risk of developing CCR. In line with this, the immune response is a key factor responsible for shaping the tumor microenvironment during carcinogenesis. Macrophage inhibitory factor (MIF) is a proinflammatory cytokine with chemokine-like functions, overproduced during cancer development, which may attract immune cells to the microenvironment, exacerbating tumor development. In this work, we determine if MIF modulates immune cell recruitment to the tumor and its capability to enhance cancer malignancy. Using male BALB/c mice WT ($MIF^{+/+}$) and Knockout ($MIF^{-/-}$), we elucidate if MIF exacerbates tumor progression and malignancy during a chemical CAC model with AOM/DSS. Briefly, the clinical signs of the mice were evaluated weekly. Mice were euthanized and tumor tissue was obtained and washed. A sample was fixed for histology, the remaining tissue was separated in a single-cell suspension, and the immune cell populations were evaluated by flow cytometry. We found that $MIF^{-/-}$ exhibited less damage during CRC development with fewer tumors. Histological analysis showed an enhanced cell transformation and Goblet cell depletion in WT mice. On the other hand, fewer neutrophils, CD4+ T cells, and myeloid-derived cells were infiltrated in the tumor tissue of $MIF^{-/-}$ mice. So far, these results suggest that the absence of MIF resulted in less tumor burden and decreased cell transformation by fewer immune cells infiltrated in tumor tissue. This way, MIF could enhance tumor malignancy by increasing chemotaxis to the tumor microenvironment.

444. 059. **ANTI-TUMOR EFFICACY OF SILVER BIONANOPARTICLES IN ANAPLASTIC THYROID CANCER CELLS**

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Background: Anaplastic thyroid cancer (ATC) represents a highly aggressive form of thyroid cancer (TC) with limited effective treatment options. Metal nanoparticles, due to their versatile properties, have gained attention in various biological applications. Silver bionanoparticles (AgNPs) have shown promise as potential agents

for cancer therapy. AgNPs were previously biosynthesized by *Pseudomonas aeruginosa* culture supernatant with an important microbicide activity. However, their anti-tumor impact in ATC cells is not known.
Objectives: We explored the anti-tumor effects of biogenic AgNPs on human ATC cells.
Methods: ATC cells (8505C, C643, THJ-11T and THJ-16T cells) and non-tumor thyroid cells (NThyOri) were treated with AgNPs (0.2–1.25 pM), for 24h. Cell viability was evaluated by MTT assay and reactive oxygen species (ROS) production by FACS. Differentially expressed genes (DEGs) were identified by transcriptome sequencing (RNA-seq). The functional properties of DEGs were characterized by Reactome pathway analyses. Apoptosis in ATC cells was assessed by analyzing the expression of cleaved caspase-3 and cleaved PARP by Western blot assays.
Results: Exposure to AgNPs induced significant morphological changes in ATC cell lines, accompanied by a marked decrease in cell viability and an increase in ROS generation. Conversely, NThyOri cells showed reduced susceptibility to these effects, suggesting a selective impact of AgNPs on ATC cells. DEG analysis between control and 0.75pM AgNPs-treated 8505C cells revealed 2242 DEGs, including 1501 upregulated genes and 741 downregulated genes. Reactome pathway analysis highlighted enrichment in pathways such as “Attenuation phase” and “HSF1-dependent transactivation” following AgNPs treatment. Additionally, AgNPs significantly upregulated apoptotic markers compared to untreated controls.
Conclusions: These findings underscore the potent *in vitro* anti-tumor efficacy of biogenic AgNPs against ATC cells. The study provides insights into the potential molecular mechanisms and pathways involved in AgNPs’ therapeutic effects. Thus, AgNPs represent promising candidates for further exploration as novel therapeutic agents for ATC.

445. 84. DEPLETION OF TREGS IN VIVO REDUCES LYMPH NODE METASTASIS OF ORAL SQUAMOUS CELL CARCINOMA (OSCC) AND PROLONGS OVERALL SURVIVAL

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Background: Immunosuppression is associated with worse prognosis in OSCC and novel immunotherapies aim at rescuing anti-tumor immune response. Regulatory T cells (Tregs) are the prototypical immunosuppressive cell type, and their prevalence is correlated worse prognosis in OSCC. **Objectives:** Evaluate the impact of systemic depletion of Tregs on nodal invasion and survival in a syngeneic orthotopic model of OSCC. **Methods:** We used DEREG mice (C.B6-Tg(Foxp3-DTR/EGFP)23.2Spar/Mmjax) which allow for depletion of Treg (CD4+FOXP3+) cells induced by diphtheria toxin. Tumors were induced by injecting $5x10^4$ MOC2 cells into the floor of the mouth. Three experimental groups were performed: G1 - control / no Treg depletion; G2 - Treg depletion 7 days after tumor induction; and G3 - Treg depletion 2 days before tumor induction. Animals were followed up and euthanized when body weight loss reached 20% of baseline (humane endpoint). Tumors and lymph nodes were collected and dissociated for flow cytometry analyses of immune (T CD4+ and T CD8+) and neoplastic (CD45-CD326+) cells, and RT-qPCR was performed to determine expression of *Tgfβ*, *Il-10*, and *Tnfsf11*. **Results:** Survival analysis revealed that G3 had a median survival of 15 days, significantly longer than G1 and G2 ($p<0.0001$ and $p<0.04$, respectively). Survival of animals in G2 did not differ from that of animals in G1 ($p=0.56$). There was a trend of reduced prevalence of neoplastic cells in the lymph nodes of animals from both G2 and G3. Depletion of Tregs was associated with significant reductions in the expression of *Il-10*, *Tgfβ* and *Tnfsf11*, increased infiltration by CD4+ and CD8+ T cells, as well as a significant decrease of CD47+ neoplastic cells ($p < 0.0001$). **Conclusion:** Tregs may facilitate lymph node invasion and reduce survival in OSCC, potentially by altering the tumor microenvironment, reducing CD4+ and CD8+ T cell infiltration, and increasing immunosuppressive gene expression, including CD47.

446. 100. CYTOPLASMIC PROTEIN ASSOCIATES TO PD-L1 IN CELL SURFACE CHECKPOINT DOMAINS ASSEMBLING A NOVEL TARGET FOR ANTIBODY RESPONSE IN LONG TERM SURVIVORS OF PANCREATIC CANCER

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Pancreatic cancer ranks 6th in cancer-related deaths for men and women, and accounts for as almost many deaths as cases, due to the difficulty in diagnosis and poor response to treatment. Nevertheless, a small cohort of pancreatic ductal adenocarcinoma (PDAC) patients survive beyond 5 years. Our aim is to understand the immune response in these patients against tumor antigens, to unravel the mechanisms that allow them to survive. To achieve this, we evaluated antibody response in a cohort of PDAC long term survivors. These patients have high titers of specific antibodies against a cytoplasmic protein (ProtA), further identifying the specific B-cell epitope. We analysed ProtA localization in PDAC cell lines and found that metastasis-derived cells exhibit an increased shift of this typically intracellular protein to the cell surface membrane. Using confocal immunofluorescence microscopy, we mapped the localization of ProtA, finding it confined to specific areas of the cell membrane, assembling a cup-like structure. These novel architectures were imaged by cryo-SIM microscopy and soft X-ray tomography, identifying a peculiar extracellular membrane organization to ProtA enriched areas, which have not been described before. We evaluated the localization of checkpoint molecules in PDAC cells and found PD-L1 and CMTM6 colocalize with ProtA at this cup-like sites. Furthermore, ProtA and PD-L1 directly interact in a solid phase assay enabling us to map the interaction to a two-binding site model, implicating ProtA as a scaffold molecule that when translocated to the extracellular membrane, anchors PD-L1 at the cell surface enhancing malignancy. Finally, ProtA-specific antibodies that phenocopy the epitopes identified in PDAC long term survivors were found to block this interaction and to disturb the cell surface association of ProtA and PD-L1. This is a step forward toward the development of new strategies to treat PDAC, with the goal of improving survival and prognosis.

447. 107. LACK OF NLRP3 INFLAMMASOME IN STROMAL CELLS PROMOTES RESISTANCE TO CISPLATIN AND REDUCES CD8 T CELL INFILTRATION IN ORAL SQUAMOUS CELL CARCINOMA (OSCC)

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Background: The influence of NLRP3 inflammasome in OSCC progression and response to treatment remains unclear, especially its specific role in neoplastic and stromal (non-cancerous) cells within the tumor microenvironment (TME). **Objective:** This study investigates the influence of NLRP3 inflammasome activity in stromal cells in OSCC progression. **Methods:** We used C57BL/6 wild-type (WT) mice (n=20) and NLRP3 knockout (KO) mice in a C57BL/6 background (n=20) in a syngeneic orthotopic model of OSCC. Briefly, $5x10^4$ MOC2 cells were injected into the floor of the mouth of mice. A non-curative dose of cisplatin was administered intraperitoneally to half of the mice 7 days post-tumor induction. Animals were euthanized after 14 days and tumors and spleens were collected. **Results:** Histopathological analysis showed both WT and KO animals had moderately and poorly differentiated tumors with necrotic regions. There were no significant differences in the number of blood vessels, irrespective of cisplatin treatment or NLRP3 expression ($p>0.05$). Cisplatin significantly reduced tumor volume and mass only in WT animals ($p<0.05$). NLRP3 KO mice had larger tumors and fewer CD8+ T cells in the spleen and tumors, as shown by flow cytometry ($p<0.05$). In contrast, cisplatin-treated tumors from NLRP3 KO mice showed increased infiltration of CD4+ T lymphocytes ($p<0.05$). The lack of NLRP3 was also associated with a lower percentage of CD4+IL-4+ cells in the spleen. Cisplatin treatment led to decreased expression of CD47 by tumor cells, suggesting increased efferocytosis activity and impaired immunosuppression in the TME. **Conclusion:** Lack of NLRP3 activity in stromal cells promotes resistance to cisplatin without affecting tumor growth or vascularization. NLRP3 activity in stromal cells is important for CD8+ T cell infiltration in the tumor microenvironment.

448. 128. BIOINFORMATIC ANALYSIS OF RNASEQ DATA REVEALS PROGNOSTIC IMMUNE HETEROGENEITY AND POTENTIAL IMMUNOTHERAPY TARGETS IN LUMINAL BREAST CANCER

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Background: Breast cancer (BC) is a heterogeneous disease, which are molecular classified on 5 intrinsic PAM50 subtypes. The Luminal subtypes A and B include around 70% of BC patients and they generally have a better prognosis, but their immunogenicity varies, potentially affecting patient outcomes. This study investigates the immune landscape of Luminal A and B breast cancer to uncover its prognostic value and potential immunotherapy. Methods: We analyzed mRNA-sequencing and clinical data from 769 non-metastatic, treatment- naive Luminal A and B patients using the TCGA dataset. Immune infiltration was assessed with the ESTIMATE algorithm, and immune cell fractions were quantified using quanTIseq. Patients were classified into high and low immune infiltrate groups based on immune scores, and survival outcomes were compared using Kaplan-Meier curves and Log-Rank tests. The Chi-squared test examined PAM50 subtype distribution, while multivariate Cox models assessed the independence of immune groups as prognostic factors. Differentially expressed genes (DEGs) between immune groups were identified using DESeq2, and enriched pathways were analyzed through Gene Set Enrichment Analysis (GSEA). All analysis were performed using R programing language. Results: Higher immune scores and CD8+ T cell infiltration were linked to better prognosis. Kaplan-Meier analysis showed a better survival profile in the high immune groups compared to low immune group ($p<0.05$), sustained in multivariate analysis (Hazard Ratio: 1.8, $p<0.05$). High immune infiltration was associated with significatively greater abundance of B cells, CD4+ T cells, Tregs, CD8+ T cells, and macrophages, while NK cells, dendritic cells, and neutrophils were less abundant ($p<0.05$). DEG analysis identified 177 DEGs, including targetable immune checkpoint genes like CTLA4 and PD1. GSEA revealed immune activation pathways enriched in high immune groups and DNA repair related pathways enriched in low immune group. Conclusion: This study underscores the prognostic importance identifying of immune infiltration in Luminal breast cancer, suggesting that patients with high immune infiltration may benefit from immunotherapy.

449. 131. EFFECTS OF SENESCENT FIBROBLASTS ON NK CELL FUNCTIONALITY, MACROPHAGE POLARIZATION AND TUMOR MICROENVIRONMENT

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Aging is associated with the accumulation of senescent cells, higher incidence of cancer and suboptimal immune responses. Thus, this work aimed to analyze how senescent cells affect Natural Killer (NK) cells and macrophages. Senescent fibroblasts (SenFb) were generated by treating the IZA mouse fibroblast cell line with 1 micromolar etoposide for 48h. Splenocytes from BALB/c mice were cultured with SenFb, control fibroblasts (ConFb) or their conditioned media (CM) with IL-12, IL-15, and IL-18 to assess the frequency of IFN-gamma-producing NK cells ($CD3^-NKp46^+$ cells) by flow cytometry (FC), or with bone marrow-derived macrophages in M1-, M2- and tumor associated macrophage (TAM)-like-polarizing conditions for 48h (M1: LPS+IFN- gamma; M2: IL-4+IL-13, TAM-like: CM from CT26 tumor cells) to assess macrophage polarization markers (M1: CD86 and CD274; M2: CD206 and MARCO) by FC. SenFb and their CM led to decreased frequencies of IFN-gamma-producing NK cells (mean±SEM SenFb: 23±6, ConFb: 35±5, n=11, $p<0.0001$; SenFb CM: 27±7, ConFb CM: 45±7, n=6, $p<0.0001$) and increased expression of CD206 and MARCO and decreased expression of CD274 and CD86 in macrophages, as assessed by FC. When $2.2x10^5$ CT26 cells were coinjected with $1x10^6$ SenFb or ConFb into 10 weeks-old BALB/c mice and tumor growth was assessed, we observed that coinjection with SenFb resulted accelerated tumor growth (day 12: $p<0.0001$), with tumors that contained a higher percentages of $CD45^+$ cells (mean±SEM ConFb: 24±3, n=4; SenFb: 63±10, n=6; $p<0.05$) but reduced percentages of NK cells (mean±SEM ConFb: 10±3, n=4; SenFb: 3±1, n=6; $p<0.01$). These mice presented higher percentages of splenic NK cells (mean±SEM ConFb: 3.3±0.2, n=5; SenFb: mean 5.3±0.8, n=6; $p<0.05$) with downregulation of NKG2D (mean±SEM ConFb: 1117±45, n=5; SenFb: 653±156, n=6; $p<0.05$) and upregulation of PD-1 (mean±SEM ConFb: 30±5, n=5; SenFb: mean 216±67, n=6; $p<0.05$). Therefore, SenFb may promote tumor growth affecting NK cell function and macrophage polarization.

450. 133. **TMEM176B AS A NEW INTRINSIC CHECKPOINT OF TH17 CELLS: POTENTIAL IMPLICATIONS IN CANCER IMMUNOTHERAPY**

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T helper 17 (Th17) cells show a remarkable heterogeneity and plasticity, with two subpopulations identified: regulatory Th17 (regTh17) and effector Th17 (effTh17) cells. However, the molecular basis of how, when, and why Th17 cells differentiate into effector or regulatory populations, as well as their *in vivo* relevance, are still poorly understood. *Tmem176b*, a druggable immunoregulatory ion channel first described by our team, is one of the few genes directly regulated by RORγt. We have found that *Tmem176b* is over-expressed in regTh17 compared to effTh17 cells, in agreement with its immunoregulatory properties. Based on these findings, we speculated that TMEM176B intrinsic expression might control Th17 cells differentiation. We evaluated differences between WT and *Tmem176b*$^{-/-}$ Th17 cells by single-cell RNA- sequencing (scRNAseq), flow cytometry and Seahorse analysis.

scRNAseq analysis revealed four clusters among WT and *Tmem176b*$^{-/-}$ regTh17 cells. Differential gene expression and pathway analysis led us to identify “stem-like”, “regulatory”, “transitional” and “effector-like” clusters, with the later being enriched in *Tmem176b*$^{-/-}$ compared to WT cells. Furthermore, *Tmem176b*$^{-/-}$ cells showed over- expression of effector-related genes such as *Nkg7, Gzma, Ifng, Runx1, and Irf3.* This effector-like signature was confirmed by *in vitro* experiments. *Tmem176b*$^{-/-}$ regTh17 cells secreted considerably more IFN-gamma, and functional evaluation showed a decreased regulatory capacity compared to WT cells. Glycolysis and HIF-1alpha signaling pathway were found to be up-regulated in *Tmem176b*$^{-/-}$ regTh17 compared to WT cells. Moreover, co- culture of *Tmem176b*$^{-/-}$ regTh17 cells with *in vitro* generated exhausted CD8^{+} T cells resulted in fewer terminal exhausted CD8^{+} T cells compared to WT cells. Furthermore, *in vivo* inhibition of TMEM176B enhanced the antitumor activity of anti-PD1 in an *Il17a*-dependent manner, increasing effTh17 and decreasing regTh17 cells within the tumor microenvironment. In conclusion, this study identifies TMEM176B as a new intrinsic regulator of Th17 cells and a potential immunotherapeutic target to pharmacologically modulate these cells.

451. 150. **A PROSPECTIVE STUDY OF BIOMARKERS ASSOCIATED TO RESPONSE TO BACILLUS CALMETTE-GUERIN (BCG) TREATMENT OF PATIENTS WITH NON-MUSCLE INVASIVE BLADDER CANCER (NMIBC). ANALYSIS OF URINE SIMPLES**

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Intravesical BCG is the gold-standard adjuvant therapy for NMIBC, but since the response rate is ~60%, biomarkers are needed to predict outcomes. We previously defined a Th2-score associated with BCG response in pre-BCG biopsies, combining GATA-3+ (Th2)/T-bet+ (Th1) ratio with EPX+ eosinophils’ density/activation by IHC. We hypothesized that a high Th2-score (HS) would be associated with better outcomes due to Th1 polarization by BCG, whereas a low Th2-score (LS) might indicate an immunosuppressive tumor microenvironment (TME), reducing BCG efficacy. We initiated a prospective study to validate the Th2-score and assess urinary lymphocytes (UL), reflecting TIL populations, using flow-cytometry, and cytokines/chemokines quantification by

multiplex assay, in samples obtained throughout treatment. Data were analyzed using Mann-Whitney or Mixed-Effects models (Prism 10.0). NMIBC patients receiving adequate BCG (induction + 3 maintenance)(n=20) were included; HS=9(favorable), LS=1(unfavorable). In urine, PMNs were the predominant population, with CD3+ T cells recoverable after 3-4 BCG doses, mostly CD4+ > CD8+, and <10% were T-γδ cells and NK/NKT. Post-induction, HS-patients showed higher proportions of CD4+PD1+, CD8+PD1+, and CD4+CD103+CD39+PD1+ cells ($p<0.05$), suggesting earlier T-cell activation. Following induction, HS-patients had higher proportions of CD4+CD103+ ($p<0.05$), with CD8+ T cells showing higher T_{EFF} and lower T_{TEMRA} frequencies. Higher levels of urinary pre-BCG IFNγ, CXCL9, MIP-1β, IL-13, and IL-2 were found in LS vs HS-patients, showing different TME polarization. Consistent with our hypothesis, BCG induced Th1-polarization in HS-patients, as indicated by increased urinary pro-inflammatory molecules such as IFNg, IL1b, TNFa ($p<0.05$) after BCG induction. In vitro stimulation of UL with BCG showed TNFα production by NK, T-γδ, CD4+, and CD8+ cells. For one patient with compatible HLA, UL stimulated with J82 bladder cancer cells ± BCG exhibited TCD4+ and TCD8+ cytotoxicity(CD107a), also seen in NK, NKT, and T-γδ cells, which additionally showed IFNγ production. These results should be correlated with clinical BCG response to establish their predictive value.

452. 151. **INHIBITION OF LACTATE SYNTHESIS IN TUMOR CELLS WITH INCOMPLETE O-GLYCOSYLATION INCREASES PD-L1 DETECTION**

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Cancer is a leading cause of death, with lung cancer being the most diagnosed worldwide. Adenocarcinomas exhibit abnormal glycosylation that favor tumor aggressiveness. We have developed a preclinical lung cancer model with aberrant glycosylation using the murine LL/2 lung tumor cell line (LL/2-Tn+) and demonstrated that Tn expression is associated with the recruitment of IL-10^+ regulatory T cells to the tumor and an increased tumor growth characterized by higher PD-L1 expression by tumor cells. Interestingly, glucose metabolism can favor lactate accumulation and promote tumor growth and immune evasion by PD-L1 expression on tumor cells. The aim of this work was to study glucose metabolism and its relation to PD-L1 expression in a lung cancer model with aberrant glycosylation. We evaluated expression of PD-L1 and metabolic enzymes in lung tumor cells treated with an inhibitor of lactate production (oxamate) to determine how modulating these pathways
modify PD-L1 expression, cell glycosylation and glucose metabolism. Glycans were analyzed by flow cytometry using lectins. We assessed glycolytic flux and oxidative capacity of tumor cells in the presence of aberrant glycosylations. Treatment with oxamate increased the detection of PD-L1 protein and modified cell glycan profile both in vitro and in vivo, as well as gene expression and metabolism of tumor cells in vitro. Furthermore, LL/2-Tn+ cells had an altered metabolism compared to its parental line. Additionally, we found that oxamate treatment influenced the growth of both cell lines in vitro and in vivo.

453. 153. **INVESTIGATION OF DIETARY INFLUENCE ON LIPID DROPLETS IN COLON CANCER DEVELOPMENT**

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Gastrointestinal cancers are highly prevalent worldwide, strongly influenced by individual habits, especially diet. Technological advances in food production have promoted greater consumption of high-fat and high-sugar foods, leading to increased calorie intake and lipid accumulation in cells. This lipid accumulation alters metabolic pathways linked to poor tumor prognosis. Altered lipid metabolism in cancer cells involves the modulation of metabolic regulators, presenting potential targets for anti-cancer interventions. This study aims to identify the role of dietary patterns in lipid droplet (LD) profile and frequency in tumor progression in vivo and in vitro. C57BL/6J mice underwent colorectal carcinogenesis induction with azoxymethane

and 3 cycles of dextran sulfate sodium (DSS), followed by three differents diets: control (Chow), high-fat and high-sucrose (HFHS), and fast-food mimicking diet (FFMD). Mice on HFHS and FFMD diets showed altered ipGTT post-tumor induction, indicating diet- induced insulin resistance. Additionally, liver discoloration, suggesting possible hepatic steatosis, and spleen enlargement were noted. Thirty days post-DSS cycles, an increase in tumor number and size were observed in high-calorie diet groups. Interestingly, mesenteric lymph node size decreased in HFHS and FFMD groups. Flow cytometry revealed no difference in B and T cell percentages across diets, but an increase in PD-1 and decrease in Tim3 in HFHS mice was observed. In vitro assays using non-tumor intestinal cells (IEC-6) and colon tumor cells (CACO-2) treated with oleate and palmitate for 24h, 48h, and 72h showed no viability change but increased LD number and size at 24h, with a subsequent decrease by 48h and 72h in CACO-2 cells. These results suggest tumor metabolism may be more adaptable in energy source utilization. Further assays to analyze the inflammatory tumor microenvironment are under evaluation to assess the evolvement of LDs in modulate tumor progression.

454. 156. **GPER ACTIVATION INDUCES DIVERGENT EFFECTS ON APOPTOSIS AND CELL MIGRATION IN A CERVICAL CANCER CELL LINE: A MARKER OF GOOD PROGNOSIS IN CERVICAL CANCER.**

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Estrogens and HPV are necessary in cervical cancer (CC) development. Estradiol increases HPV oncoproteins levels and these promote G protein-coupled estrogen receptor (GPER) expression. GPER levels increases as CC progresses. GPER has anti- and protumorigenic effects, for this reason its implication in cancer is still controversial. This study aimed to determine how GPER activation affects the transcriptome, migration and invasion of CC cells. These processes were evaluated by RNA-seq, transwell assays, and immunofluorescence in SiHa cells and non-tumorigenic keratinocytes transduced with HPV16 oncogenes E6 or E7 stimulated with the GPER-selective agonist G-1. Transcriptome analysis showed pathways enriched by G-1 in SiHa cells, these include the following processes: proliferation/apoptosis (TNF-alpha signaling via NFKB, response to UV radiation, mitotic spindle formation, G2/M cell cycle, response to misfolded proteins, IL-6/JAK/STAT), cellular metabolism (oxidative phosphorylation), and cell migration (angiogenesis, epithelial-mesenchymal transition, TGF-beta signaling). The main differentially expressed genes were PTGS2, classified as pro-tumor/anti-tumor, FOSL1, TNFRSF9, IL1B, DIO2, and PHLDA1 described as anti-tumor, together with other under-expressed genes with pro- tumor effects may inhibit proliferation. Moreover, DKK1 overexpression may indicate an inhibition of cell migration. Due to the lack of evidence available about the effect of G-1 on migration and invasion, we evaluated these processes and the expression of associated proteins. G-1 increased vimentin expression in SiHa cells and induced an opposite effect in HaCaT-16E6 and HaCaT-16E7. However, G-1 did not modify alpha-SMA expression in any of the cell lines. Furthermore, G-1 did not induce changes in cell migration in any of the cell lines, while it promoted an increase in cell invasion in HaCaT-16E7 cells. In conclusion, GPER is a good prognostic marker because it activates apoptosis and inhibits proliferation through different signaling pathways, without promoting migration and invasion in cervical cancer cells. Therefore, G-1 could be a tool in the treatment of this neoplasia.

455. 157. **HISTAMINE IMPRINTS IMMUNE CHANGES IN THE MICROENVIRONMENT AND TUMOR GROWTH BY INTERACTING WITH THE H4 RECEPTOR**

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Histamine (HIS) plays a key role in inflammation

through four G-protein-coupled receptors (H1R-H4R). Elevated levels of HIS and its receptors were described in several tumors. Previously, we proved that HIS acting on dendritic cells enhances cross-presentation of the soluble antigen ovalbumin and induces the activation of non-conventional CD8 T cells. Here, we studied the H4R involvement in breast cancer development with special focus on its immunomodulatory role. Regarding this, we used *in vitro* and *in vivo* the 4T1 murine breast cancer cell line and the HIS antagonist JNJ-7777120 (JNJ; 10 µM) to specifically block HR4. The crystal violet colorimetric assay showed that 72-h JNJ treatments inhibited cell proliferation (JNJ 0.64±0.10 *vs*. vehicle 0.97±0.04; n
= 7), which correlated with G0/1 cell cycle phase arrest (JNJ 47.7±4.2 *vs*. vehicle 37.3±6.2; n = 4), assessed employing propidium iodine and flow cytometry analysis. This antagonist also increased the expression of PD-L1 ($p < 0.01$, n = 7) and MHC class I ($p < 0.05$, n = 8), while significantly decreased FAS levels ($p < 0.01$, n = 7), as determined by flow cytometry after 24-h treatments. Furthermore, the subcutaneous administration of JNJ-treated 4T1 cells (10^5) in BALB/c mice was accompanied by a significant reduction ($p < 0.01$) of tumor mass respect to vehicle control on day 14 post-inoculum (JNJ 463.9±232.7 *vs*. vehicle 707.1±189.7; n > 15). Tumors originated from JNJ-treated cells presented a substantially increased ($p < 0.05$; n = 10) infiltration of lymphocytes CD8+ on day 7. However, such difference was not observed on day 14. Strikingly, ELISA analysis of various cytokines showed elevated levels of IFN-γ and IL-10 ($p < 0.05$; n = 10) in cells from JNJ-treated tumors. In conclusion, our results suggest that HIS interacting with H4R has a dual effect on both tumor development and the immune microenvironment.

456. 158. **CANCER-ASSOCIATED FIBROBLASTS PROMOTE NEUTROPHIL ACTIVATION TOWARD A FUNCTIONAL PROTUMOR PHENOTYPE IN CERVICAL CANCER PROGRESSION**

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Background: Tumor microenvironment (TME) influences cervical cancer (CC) progression where the most abundant cells are cancer-associated fibroblasts (CAFs) and tumor-associated neutrophils (TANs). The presence of fibroblasts expressing CAFs markers (vimentin, αSMA, and FAP) during CC development stages has been associated with tumor progression. TANs may exert a dual role in cancer either anti- or pro-tumor through their cytotoxic activation and cytokine production which play an important role in the development of CC. Thus, understanding the complex interplay between CAFs and TANs and their tumor microenvironment has the potential to identify TANs as viable therapeutic targets in CC progression. **Objectives:** This study aimed to evaluate the effect of CAFs derived from different stages of cervical cancer on the functional polarization and activity of neutrophils. **Methods:** Vimentin, αSMA, and FAP expression of CAFs derived from samples of different clinical stages of cervical cancer were assessed by immunofluorescence. Subsequently, neutrophils from clinically healthy subjects were stimulated with supernatant from CAFs (CSN) to assess cytokine profiles by multiplex ELISA, mitochondrial metabolic activation by MTT assay, and ROS production by H2DCFDA assay, finally neutrophil functional phenotype by expression of activation markers CD66b, neutrophil elastase (NE) and MMP9 by immunofluorescence. **Results:** Fibroblasts derived from samples of different clinical stages of CC-expressed CAF markers. It was observed that CSN of high grades of CC promoted greater metabolic activation and higher ROS production in neutrophils. Cytokines secreted by neutrophils stimulated with CSN increased the production of proinflammatory cytokines. Finally, neutrophils stimulated with CSN showed a greater expression of activation markers CD66b, NE, and MMP9. **Conclusion:** CAFs promote increased neutrophil activation in accordance with tumor progression, which may lead toward a functional pro-tumor phenotype of neutrophils in cervical cancer.

457. 172. **IMPACT OF EXTRACELLULAR ACIDOSIS ON THE PHENOTYPE AND FUNCTION OF NATURAL KILLER CELLS AND IMPLICATIONS FOR THEIR ANTITUMOR**

ACTIVITY

Fanny Wandersleb[1], María Sofía Amarilla[1], María Natalia Rubinsztain[1], Mariana Gantov[1], María Cecilia Santilli[1], María Victoria Regge[1], Adrián David Friedrich[1], Carolina Inés Domaica[1], Mercedes Beatriz Fuertes[1], Norberto Walter Zwirner[1]
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Upon encountering tumor cells, natural killer (NK) cells become activated and display effector functions such as cytotoxicity against these target cells and cytokine secretion (mainly IFN-γ). However, several factors within the tumor microenvironment, including extracellular acidosis promoted by the growing tumor, can impact on NK cell effector functions. As the effect of extracellular acidosis on NK cells remains ill-explored, our aim was to determine whether the acidic pH affected NK cell activation and functions. Peripheral blood mononuclear cells (PBMC) isolated from healthy donors were stimulated with IL-12, IL-15 and IL-18 and cultured for 24 h in a pH=7.2 or a pH=6.5 complete medium. To assess the degranulation (frequency of $CD107^+$ cells), K562 cells were added for the last 5 hours. Thereafter, the frequency of IFN-γ-producing, CD25 (IL-2Rα) expressing NK cells ($CD3^-CD56^+$ cells) and degranulation was assessed by flow cytometry (FC). Culture in the acidic medium led to a significantly lower frequency of IFN-γ^+ NK cells (mean±SEM in pH 7.2: 55.5±6.6; in pH 6.5: 32.3±11.7, $p<0.01$, two-way ANOVA), a lower frequency of $CD25^+$ NK cells (mean±SEM in pH 7.2: 62.8±4.8; in pH 6.5: 48.2±7.8, $p<0.05$, paired t-test) and of NK cell degranulation (mean±SEM in pH 7.2: 50.6±6.2; in pH 6.5: 28.6±6.8, $p<0.001$, two-way ANOVA). Culture for 24 h of NK cells previously cultured in acidic medium for 24 h led to a recovery in the frequency of IFN-γ-producing NK cells (mean±SEM in pH 7.2 for 48h 56.0±8.0, pH 6.5 for 24h + pH 7.2 for 24h 46.7±8.3) and of the frequency of NK cell degranulation (mean±SEM in pH 7.2 for 48h 55.2±5.8, in pH 6.5 for 24h + pH 7.2 for 24h 44.8±5.0). These results suggest that acidic pH, typical of the TME, attenuates NK cell activation and function, such as degranulation and IFN-γ production in a reversible manner.

458. 179. **TUMORAL PD-L1-DEPENDENT REGULATION OF TAM IMMUNOSUPPRESION DURING TNBC PROGRESSION**

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T-cell PD-1 engagement by tumoral PD-L1 is widely recognized as one of the main immunosuppressive mechanisms driving cytotoxic T-cell exhaustion. However, it is unclear how tumoral PD-L1 modulates immune evasion in non-T cell PD1+ immune populations, as tumor-associated macrophages (TAMs). To interrogate this, we generated a PD-L1 KO TNBC-like tumor model in the murine EO771 cell line using CRISPR/Cas9 editing, allowing us to profile the immune infiltrates of the tumoral microenvironment (TME) *in vivo* during tumoral progression. Using flow cytometry (FC) to characterize the immune infiltrates of early vs late-stage WT tumors, we found a late-stage decrease in F480+ CD206+ populations, suggesting that M2 TAM polarization is inhibited during tumor development. Furthermore, analyzing PD-1 expression of immune infiltrates we observed an increase in PD1+ M2 TAMs at late-stage, suggesting that advanced tumors are more responsive to PD-L1. In addition, examining PD-L1 KO vs WT tumors at early and late-stage we found that tumoral PD-L1 inhibits M2 TAM polarization exclusively at late stage. Using bone marrow-derived macrophages in tumoral cell & conditioned media co-culture experiments, we found that M2 TAM inhibition is a direct effect that involves tumor cell- to-macrophage contact. Interestingly, M2 TAMs from late-stage WT tumors showed increased MHCII+ expression, suggesting an improvement in antigen-presentation potential. Moreover, using FC to analyze GFP+ tumor cell phagocytosis, we found that tumor progression triggers phagocytosis exclusively in M2 TAMs. Comparing PD-L1 KO vs WT tumors, we observed that tumoral PD-L1 inhibits TAMs phagocytosis both *in vivo* and *in vitro*.

All together, these results suggest that M2 TAMs acquire anti-tumoral features during tumor progression and that tumoral PD-L1 dependent inhibition of M2 polarization plays a critical role in TAM immunosuppression during late stages of TNBC progression.

459. 186. **5-FU CAUSES CHANGES IN THE STEMNESS PHENOTYPE AND IN CD59 LEVELS, MODIFYING CETUXIMAB ACTIVITY IN COLON CANCER CELLS (SW480-SORE6)**

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Colorectal cancer (CRC) ranks third in incidence and second in mortality worldwide. The treatment involves the use of cytotoxic and targeted agents. Patient survival has improved with the use of monoclonal antibodies (mAb); however, their efficacy may be limited by membrane-bound complement regulatory proteins (mCRP), such as CD59, which can inhibit complement-dependent cytotoxicity (CDC). Although the impact of mCRP expression on mAb efficacy is well known, its relationship with cytotoxic therapy and cancer stem cells (CSC) remains unexplored. CSC are a population within tumors with stem properties, such as self-renewal and pluripotency, and are responsible for chemoresistance and tumor initiation. CD59 can regulate evasion of apoptosis, chemoresistance, and tumor progression through intracellular signaling, and SOX2 can regulate its expression. 5-FU can inhibit the expression of CSC markers, and even though it has been used in CRC for 40 years, its relationship with CSC and mCRP has not been elucidated. Thus, we analyzed how the therapy modulates the expression of these proteins and its impact on CSC-like cells in a colon cancer cell line (SW-480) transduced with a reporter system (SORE6-GFP) that responds to the presence of SOX2/OCT4. Transduced cells were sorted into GFP+ and GFP- cell populations, and their biological behavior was characterized. The stemness phenotype was analyzed using a sphere formation assay. Membrane protein expression levels were measured using flow cytometry. Interleukin levels were determined by ELISA. GFP- cells showed CSC-like properties when cultured under sphere conditions. 5-FU inhibits sphere formation and stem cell marker levels in both GFP+ and GFP- cells and induces changes in CD59 levels, which affect cetuximab-induced CDC. EGFR levels are associated with sensitivity to cetuximab- mediated CDC. CD59 silencing will clarify its involvement in our findings.

460. 187. **PIOGLITAZONE MIGHT MODULATE CAF-TUMORAL CELLS CROSS-TALKING REGULATION OF TUMORAL MICROENVIRONMENT IN AN IN-VITRO MODEL**

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Background: Cancer-associated fibroblasts (CAFs) are key elements in tumoral microenvironment, play several functions such as promotion of growing and migration of tumoral cells and induction of extracellular matrix remodeling. Cross-talking between CAFs and tumoral cells play a crucial role in cancer progression, therefore, CAFs have become a potential target to improve cancer therapies. Pioglitazone has shown antifibrotic and anti-inflammatory activities, decreasing the expression of IL-1, IL-6, TNF-alpha, alpha-SMA and TGF-beta. For this reason, pioglitazone could be promising in the treatment of cancer by inhibition of CAFs **Objective:** Evaluate the *in-vitro* effect of pioglitazone on CAFs and tumor cell line HeLa cross-talking. **Methods:** CAF were stimulated with HeLa supernatant (HSN) and vice versa (CSN), both with and without pioglitazone for 24hrs, control and vehicle were also included. Gene expression of alpha-SMA, FAP, IL-6, IL10 and TGF-beta1 was evaluated by RT-PCR. ERK1/2 phosphorylation was evaluated by western blot and protein level of alpha-SMA and FAP was evaluated using immunofluorescence. Cytokine secretion was evaluated by ELISA. Migration was assessed by wound healing assay. ROS production was measured with H2DCFDA-ROS assay. Mitochondrial metabolism was evaluated by MTT assay and indirect glucose uptake was measured by glucose oxidase colorimetric method. GraphPad software was used for statistical analysis. **Results:** Pioglitazone reduces

the mRNA expression of alpha-SMA, increase the expression of IL-6 at mRNA but not protein, reduces ERK1/2 phosphorylation, inhibits cell migration, mitochondrial metabolism and ROS production in CAF stimulated with HSN. In HeLa cells stimulated with CSN, pioglitazone inhibit migration, increase the secretion of IL-8, decrease mitochondrial metabolism, reduces ROS production and decrease glucose uptake. **Conclusions:** Pioglitazone reduce CAF activation, reduces the ROS production, decrease glucose uptake and cell migration. However, in-vivo assays are needed to determine its role in tumoral microenvironment and if the increment of IL-8 is beneficial or not in cancer treatment.

461. 188. **NEUTROPHIL EXTRACELLULAR TRAPS (NETS) DRIVE A CHEMORESISTANT PHENOTYPE IN HUMAN BREAST CANCER CELLS THROUGH PI3K / AKT / NF-KAPPAB PATHWAY**

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Background: Neutrophil Extracellular Traps (NETs) are chromatin structures decorated with proteins produced by neutrophils. In cancer, NETs have been linked to multiple steps of tumor progression. We demonstrated that NETs promote a pro-metastatic phenotype in non-aggressive breast cancer cells through the epithelial-mesenchymal transition (EMT) process. EMT has been linked with chemoresistance, but NETs' role in this process is obscure. **Objectives:** Investigate NETs' influence on *in vitro* chemoresistance acquisition by human breast cancer cells. **Methods:** MCF7, T47D and MDA-MB-231 were used. Neutrophils isolated from healthy donors' blood were stimulated with phorbol 12-myristate 13-acetate, producing NETs. Cells were treated with NETs for 24h. Occasionally, PI3K, AKT, or NF-kB inhibitors were used 1h before NETs treatment. Also, AKT knockdown was performed for 48h in MDA-MB-231 cells using reverse transfection protocol with lipofectamine RNAiMAX. *BCL-2* and *BAX* expression levels were measured by qPCR. NETs' impact on responses to doxorubicin was evaluated by Colony Formation (CF), MTT, and Western Blot (WB) for apoptotic components assays. For 3D culture, cells were seeded in ultra-low attachment plates with 2% Cultrex Basement Membrane Extract, in the presence or not of NETs and doxorubicin. Spheroids were photographed, and viability measured by PrestoBlue assay over 10 days. **Results:** All models showed that NETs were not cytotoxic to the cells. NETs treatment improved CF ability and protected all cells against doxorubicin, observed by CF, MTT, and WB assays. NETs' treatment upregulated Bcl-2's mRNA expression (antiapoptotic protein) while downregulating Bax's expression (proapoptotic protein). MCF7 and T47D spheroids with NETs exhibited increased viability and resistance to doxorubicin. PI3K, AKT, NF-kB inhibitors, or knockdown of AKT prevented NET's protective effects. **Conclusion:** NETs promote a chemoresistant phenotype in breast cancer cells, impacting responses to doxorubicin in 2D and 3D models via PI3K/AKT/NF-kB pathway. Our data reveal new strategies to overcome chemotherapy resistance in breast cancer.

462. 189. **IN SILICO ANALYSIS OF SINGLE CELL RNA SEQUENCING DATA IDENTIFIES MACROPHAGE POPULATIONS IN BREAST CANCER THAT DIFFER FROM THE M1AND M2 PARADIGM**

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Tumor-associated macrophages (TAM) are a central component of the tumor microenvironment. TAMs mediate both anti- and pro-tumoral processes and its abundance correlates with patient prognosis, especially in (breast cancer) BRCA patients. The conventional M1/M2 paradigm, categorizing macrophages as anti- or pro-tumoral, proves insufficient to capture the TAM heterogeneity identified in different types of cancer through advanced technologies like single-cell RNA sequencing (scRNA-seq). Our study aims to unravel the intricacies of TAM heterogeneity and its potential impact on clinical outcome. We uti-

lized the R programming language and conducted unsupervised analyses, including weighted gene co-expression network analysis, to identify highly correlated genes. This approach was applied to publicly available scRNA-seq datasets, which include samples from blood, tumor, and non-tumoral mammary tissue (NT tissue) from both cancer patients and healthy individuals. Contrary to the traditional M1/M2 classification, our analysis identified eight distinct gene signatures, each corresponding to different TAM populations with unique functional profiles. Among the most notable functions are high interferon-genes response, antigen presentation, phagocytosis, and lipid metabolism. Importantly, these findings were consistently observed across two independent scRNA-seq BRCA datasets. These TAM signatures exhibited differential enrichment in either blood monocytes or macrophages from NT tissue, with a differentially expressed gene signature (e.g. *CXCL9*, *FOSB*, IL-1 beta) in peripheral monocytes from breast cancer patients compared to those from healthy individuals. Importantly, we found that TAMs with high expression of interferon response genes were associated with a favorable prognosis, whereas phagocytic TAMs were linked to a poor prognosis in BRCA patients, based on data from a cohort of 1,100 individuals. Notably, none of the identified TAM populations perfectly aligned with a conventional M1 or M2 phenotype. These findings underscore the inadequacy of the M1/M2 paradigm in capturing the complexity of TAM and emphasize the necessity for additional research to comprehensively characterize TAM heterogeneity.

463. 190. **MIR-3065-5P ASSOCIATED WITH PROINFLAMMATORY CYTOKINES REGULATION: IMPLICATIONS IN THE PROGNOSIS OF CLINICAL OUTCOME IN COLORECTAL CANCER (CRC)**

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Background: The prognosis of Colorectal cancer (CRC) is mainly based on clinical stage; however, CRC is considered a complex disease due to its molecular heterogeneity. The development of molecular biomarkers that facilitate the diagnosis, and prognosis of patients remains fundamental. Previously, miR-3065-5p was established as a regulator of proinflammatory cytokines in CRC. However, its clinical relevance remains unclear. **Objective:** To determine the clinical utility of the miR-3065-5p expression levels in a Mexican cohort of CRC patients. **Methods:** A cohort of 49 CRC patients from the National Cancer Institute of Mexico was included to collect clinical and miRNA expression data. The expression of miR3065-5p was compared between CRC and non-tumoral adjacent tissues. Prognosis assessment considering miRNA expression levels was tested using Kaplan–Meier survival curves and Cox regressions. Statistical significance was defined as $p \leq 0.05$. **Results** The miR-3065-5p expression was different between non-tumoral adjacent and tumoral tissue (p=0.02). The miRNA levels predict the overall survival (OS), patients with low expression had a median OS of 70 months, while patients with high levels did not reach median-OS (p=0.041). Male patients with low expression of this miRNA had an OS of 70 months whereas patients with high levels did not reach the median-OS (p=0.050). At uni-multivariate analysis, clinical stage (HR: 1.30, CI 1.23 – 2.30; p: 0.001) and low levels of miR-3065-5p (HR: 1.30, Ci 1.23 – 2.30; p: 0.001) remain as predictor factors of OS. Finally, we designed “Prognosis miRNAs assessment in cancer” (PROMIR-C) that integrates clinical features with miR-3065-5p expression levels. **Conclusion** These findings support the clinical utility of miR-3065-5p in the diagnosis and prognosis of CRC. PROMIR-C is a fundamental tool for clinicians in treatment decision-making, prognosis assessment, and outcome of CRC.

464. 198. **PHENOTYPIC CHARACTERISTICS OF INFILTRATING CD8+ VIRTUAL MEMORY T CELLS IN MURINE TUMOR MODELS**

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Virtual memory $CD8^+$ T cells (TVM) are a subset of T cells exhibiting memory phenotype in the absence of prior antigen exposure carrying a

TCR repertoire as diverse as naïve T cells (TN). TVM are highly functional as conventional antigen (Ag)-experience memory T cells (TMEM), however, they rather respond to cytokine stimulation, such as interleukin (IL)-12, IL-18 and IL-15 than TCR signals. Currently, only a few studies have explored TVM cells in the tumor microenvironment, and it remains unclear whether their infiltration is tumor- dependent and influenced by tumor antigens.

To address these issues, we developed an experimental model using two murine tumor cell lines, B16-OVA (melanoma) and MCA-OVA (fibrosarcoma), expressing the ovalbumin (OVA) protein. B16-OVA represents an immune- infiltrated "hot" tumor, while MCA-OVA is characterized as a poorly infiltrated "cold" tumor. C57BL/6 WT mice were subcutaneously injected with either B16-OVA or MCA-OVA cells in the right flank. Tumors were harvested at days 12 or 24 and tumor-infiltrating $CD8^+$ T cells were analyzed by flow cytometry, focusing on TN, TVM, and TMEM. Although TMEM predominated in both tumors, the infiltration of TVM cells was significantly higher in MCA-OVA compared to B16-OVA, suggesting a tumor-dependent infiltration of TVM cells. Furthermore, the analysis of infiltrating TVM OVA^+ cells demonstrated that the presence of the cognate Ag in the tumors do not determine that TVM cells will acquire a TMEM phenotype.

Interestingly, TVM cells acquire a more cytotoxic phenotype in the tumors ($CD62L^{lo}$ $KLRG1^{pos}$ $NKG2D^{hi}$) than TVM present in the corresponding draining lymph nodes ($CD62L^{hi}$ $KLRG1^{neg}$ $NKG2D^{lo}$). These data encourage further investigation into the signals that drive TVM cell migration to different tumor microenvironments, as well as the potential cytotoxic capacity of infiltrating TVM cells in experimental cancer models.

465. 199. **EXTRACELLULAR VESICLES DERIVED FROM LYMPH NODE AND PLASMA IN B CELL LYMPHOMA**

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Background: B-cell lymphomas (BCL) constitute nearly 95% of all lymphoma cases. Extracellular vesicles (EVs) are heterogeneous structures that act as intercellular messengers. However, few studies have shown their secretion by human tissues, and none have examined their presence in lymph node explants from BCL. Aims: -To characterize EVs derived from lymph node (tEVs) and plasma (pEVs) of BCL and healthy donors (HD). -To determine the binding of Rituximab (anti-CD20, RTX) to pEVs expressing CD20 antigen. Methods: tEV were isolated through ultracentrifugation, while pEVs were isolated by (SEC). Characterization included western blot (WB), flow cytometry (FC), nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM). IC formation was confirmed by protein A–immunogold labeling. Plasma C4 complement levels were measured by turbidimetry. Results: Isolated tEVs and pEVs from BCL and HD exhibited: enrichment of EV markers CD81/CD107a. As expected, BCL tEVs had higher CD20 expression (mean 50.87% ± 21.46, n=3) compared to HD tEVs (n=3, $p<0.01$) by FC. Notably, there was a significant correlation ($p<0.01$, $r=1$) between the frequency of CD20+ cells in tissue by FC and the percentage of CD20+ tEV-positive beads. Notably, BCL pEVs had higher CD20 expression compared to HD pEVs, evidenced by an increased CD20/CD81 index in WB (n=13). We also observed that pEvs, upon exposure to the therapeutic anti-CD20 antibody, formed IC, as revealed by TEM (n=1). There were also decreased levels of C4 component after RTX infusion. Importantly, while CD20/CD81 index decreased after RTX infusion in the early stages, it increased in the advanced stages of BCL. Conclusion: EVs released by lymph nodes are found in plasma and reflect the CD20 protein expression of the parental cells. Additionally, CD20 expression in pEVs could serve as a surrogate marker of tumor burden.

466. 202. **DEEP IMMUNE PROFILING UNCOVERS MECHANISMS OF EARLY VERSUS LATE ONSET IMMUNE-RELATED ADVERSE EVENTS INDUCED BY FIRST-LINE IMMUNE CHECKPOINT INHIBITORS IN MELANOMA AND NSCLC PATIENTS**

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Immune-related adverse events (irAEs) are significant consequences of immune checkpoint inhibitor (ICI) therapy, occurring in 10%-35% of patients, typically within the first four months (~85%). This prospective study investigates baseline immune mechanisms associated with time to onset of irAEs. Sixteen patients with advanced/metastatic melanoma or NSCLC receiving first-line ICIs were enrolled. Severe toxicities (G3-G4), according to CTCAE (v.5.0), were grouped as Early (n=9, ≤4 months) or Late (n=7, >4 months). PBMCs were analyzed using flow cytometry and scRNA-sequencing for whole transcriptome and VDJ analysis. Plasma soluble analytes were measured using a multiplex assay. Statistical significance was defined as $p<0.05$, and differentially expressed genes (DEGs) with a FC of ≥1.25 were considered significant. The median time to irAEs onset was 3.35 months (Early: 2.4 months; Late: 7.6 months). DEG analysis revealed that Early irAEs were associated with IL-1 and IL-2 signaling, T cell differentiation, chemokine/cytokine activity, and infectious/inflammatory pathways. Late irAEs were enriched for genes involved in antigen processing/ presentation and negative regulation of immune system process. Baseline flow cytometry identified CXCR5, TOX, and TIM-3 as key markers differentiating Early from Late irAEs. Early group showed increased CXCR5+ leukocytes and trend toward elevated IL-8 and IL-1β. Late irAEs were marked by higher TOX expression. These parameters may serve as predictors of irAE onset. scRNA-seq showed Early's CXCR5+ leukocytes enriched for activation and proliferation pathways, while Late's favored negative regulation. Early TOX+ cells were activation-prone, contrasting with Late's corticosteroid response and inhibition. Clonality analysis revealed distinct clonotype enrichment profile and differential CDR3 sequences. Therefore, Early irAEs were linked to a baseline activated immune profile while Late irAEs were associated with exhausted/terminally exhausted immune populations, potentially requiring the reversal of exhaustion or activation of new clones. These findings have potential implications for personalized medicine, enabling early irAE screening and optimizing treatment regimens.

467. 205. PHENOTYPE AND EFFECTOR FUNCTIONS OF TUMOR-INFILTRATING B LYMPHOCYTES IN B16F10-OVA TUMOR-BEARING MICE

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Previous studies have highlighted the crucial role of CD8+ T and NK cells in the antitumor immune response, but the role of B cells remains less clear. B cells can exert both pro– or anti–tumorigenic functions depending on their phenotype and the microenvironment in which they are found. We aim to study the phenotype and effector functions of tumor-infiltrating (TI) B lymphocytes in a murine melanoma model. C57BL/6 mice were injected intraperitoneally with 4x10□ B16F10-OVA tumor cells. On day 13, tumors, spleens, and inguinal lymph nodes were collected, and the B-cell compartment was analyzed by flow cytometry. We identified three TI-B cell subsets: unswitched B cells (72.4% ± 5.1%), activated B cells (18.0% ± 3.1%), and plasmablasts/ plasma cells (PB/PCs, 8.3% ± 4.8%). Tumor-bearing mice had higher numbers of leukocytes ($p<0.01$) and B cells ($p<0.05$) in the spleen but lower in the lymph nodes ($p<0.01$), compared to non-tumor control mice. In these tissues and in both groups studied, most B cells showed an unswitched phenotype. IgM was expressed by nearly 100% of unswitched B cells across all evaluated tissues, while IgA was present in PB/PCs only within tu-

mors. CD39 and PD-L1, molecules involved in immune regulation, were highly expressed only in TI PB/PCs. In contrast, activated and unswitched TI-B cells showed low or no expression of these markers. Ex vivo studies demonstrated TI- B cells can produce IFNγ, IL-17A, and TNFα under stimulation with PMA/ionomycin, CpG- ODN, or αIgM-αCD40-IL-4. ELISpot analysis demonstrated that TI antibody-secreting cells produce tumor-specific antibodies, predominantly of the IgM isotype, with a smaller proportion of IgA. These results suggest that TI B cells secrete antibodies and cytokines that may be involved in modulating the immune response against tumors. Further studies are needed to determine how these functions influence the overall anti-tumor immune response.

468. 212. **ACETYLCHOLINE REGULATES PDL-1 EXPRESSION IN PBMCS POPULATIONS FROM GLIOBLASTOMA PATIENTS**

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Glioblastoma multiforme (GBM) is the most common and malignant primary brain tumor in adults. Acetylcholine (ACh) is a neurotransmitter from parasympathetic nerves and a non-neuronal paracrine mediator produced by different cell types. It has been established that ACh exceeds its role as a neurotransmitter, being involved in non-neuronal systems, including the immune system and tumor progression. Previously, we demonstrated the presence of the cholinergic system in glioblastoma cell line U251 and tumor biopsies. The aim of this work is to study the impact of ACh on Programmed Death-Ligand 1 (PD-L1) expression within distinct peripheral blood mononuclear cell (PBMC) subsets in GBM patients. PBMCs from peripheral blood samples were cultured in presence or absence of ACh for 24 hours, and the expression of PD-L1 was evaluated by flow cytometry. Our results reveal a significant increase in PD-L1 expression within CD14+, and CD14+/CD16+ PBMC populations from GBM patients treated with ACh ($p<0.05$), however, we did not find significant differences in PD-L1 expression within CD16+ PBMC population. Intriguingly, healthy donors do not exhibit this relationship, showing no significant differences in CD14+, CD14+/CD16+ or CD16+ PBMCs populations, implying that ACh appears to modulate PD-L1 expression differentially within CD14+ and CD14+/CD16+ PBMCs subsets when comparing healthy donors with GBM patients. Furthermore, the analysis using the web server TIMER2.0 revealed that GBM tissue shows significantly lower expression of acetylcholinesterase (AChE) when compared to adjacent normal tissue ($p <0.01$). PDL-1 was increased by ACh, which we previously described as a mediator defining a Th2 profile. However, PDL-1 expression was not affected by the classical Th2 inducer, thymic stromal lymphopoietin (TSLP). Our results indicate that potentially increased levels of ACh in the tumor microenvironment can produce an increase in PD-L1 expression, which may lead to a mechanism of tumor escape.

469. 218. **EVALUATION OF CYTOKINES AS MEDIATORS OF DIFFERENTIAL TUMOR GROWTH OF ANAPLASTIC THYROID CANCER XENOGRAFTS**

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Background: Anaplastic thyroid cancer (ATC) represents an aggressive form of undifferentiated thyroid cancer with limited treatment options, underscoring the critical need for new therapeutic targets. Recently, we observed distinct tumor growth patterns in xenograft mouse models derived from two ATC cell lines, each harboring genetic mutations frequently associated with ATC, such as BRAF and RAS. However, the mechanisms underlying these differential growth patterns remain poorly understood. Objectives: We aimed to investigate the involvement of cytokines as mediators of differential tumor growth in ATC xenografts. Methods: Xenograft models were es-

tablished in SCID-NOD mice using ATC 8505C (BRAF) and C643 (HRAS) cell lines. Flow cytometry assessed intracellular cytokine (IL-6, IL-10, TNF) levels in tumor tissues. IL-6 expression was further analyzed by RT-qPCR and Immunofluorescence (IF). Human monocytes (THP-1 cells) were treated with conditioned media (ATC-CM) from 8505C or C643 cells to measure IL-6 secretion via ELISA. Gene expression profiles were obtained from the NCBI Gene Expression Omnibus database and analyzed using bioinformatics analysis. Results: IL-6 expression was significantly higher in C643 xenograft tumors compared to 8505C tumors, whereas IL-10 and TNF levels showed no significant differences between these models. RT-qPCR and IF analysis confirmed elevated IL-6 levels in C643 tumors compared to 8505C tumors. Additionally, C643-CM induced significantly greater IL-6 secretion in THP-1 cells compared to 8505C-CM. Analysis of public datasets revealed elevated IL-6 expression in human ATC samples versus normal thyroid tissues. Conclusions: These findings suggest that IL-6 may mediate differential tumor growth in ATC xenografts, potentially through a RAS mutation-dependent mechanism. However, the contributions of other cytokines cannot be excluded, as they may also participate in this process. Our study lays the groundwork for developing original therapeutic strategies that target specific cytokines in a genetically dependent manner for the treatment of ATC

470. 235. **TIM3 EXPRESSION IN MONOCYTES AND MACROPHAGES IN THYROID CANCER**

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Background: Anaplastic thyroid cancer (ATC) is the most aggressive form of thyroid cancer (TC). Effective therapies for ATC patients are limited, highlighting the need to identify new mediators to develop novel therapeutic alternatives. Macrophages (Ma) are a major component of ATC-infiltrating cells, and their presence is associated with a poor prognosis. We recently identified the expression of the immune checkpoint receptor TIM3 on macrophages in ATC-cell-induced xenograft tumors. Objectives: The aim of this study was to investigate TIM3 expression on monocytes (Mo) and Ma in patients with TC. Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from TC patients and healthy donors from whole blood. RNA was extracted from PBMCs and from different cell lines: 8505C, C643 (ATC); TPC1 (Papillary Thyroid Cancer, PTC); NThyOri (non-tumor thyroid cells); Jurkat (T cells) and THP-1 (human monocytes). TIM3 expression in Mo and in tumor-infiltrating Ma was analyzed by RT-qPCR and/or Immunofluorescence (IF). Results: Real time RT-PCR results and IF revealed that the TIM3 mRNA expression levels in PBMCs were higher compared to Jurkat cells and THP-1 cells. In contrast, TIM3 expression was not detectable in thyroid cells, indicating that human thyroid cells do not express significant levels of TIM3. We did not find any significant difference in the expression of TIM3 expression in PBMCs in patients with PTC. Furthermore, and in agreement with previous findings, we observed tumor-infiltrating Ma in PTC and ATC. More interestingly, we detected the expression of TIM3 in Ma in ATC by IF. Conclusions: Our results reveal, for the first time, TIM3 expression in tumor-infiltrating Ma in human ATC. This evidence suggests that TIM3 in macrophages may represent a potential therapeutic target for treating ATC patients.

471. 243. **CD39: A KEY REGULATOR OF TUMOR PROGRESSION AND A PROMISING THERAPEUTIC TARGET**

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CD8+ T lymphocytes (LiTCD8+) play a crucial role in controlling tumor growth. However, within the tumor microenvironment (TME), several factors induce T cell exhaustion impairing the effectiveness of these T cells. Although immunotherapies aim to restore the function of LiTCD8+ exhausted (LiTEx) cells, their success is limited in certain cancers, making the identification of new therapeutic targets essential. The ectoenzyme CD39 plays a pivotal role in tumor progression by modulating the TME.Its enzymatic activity, which converts extracellular ATP to immunosuppressive adenosine, contributes to the creation of a pro-tumorigenic niche. In this study, we investigated the role of CD39 in tumor progression. C57BL6 (WT) and CD39 knockout (CD39KO) mice were subcutaneously injected with either 1x10□ B16F10-OVA or 0.5x10□ MC38 tumor cells. Tumors were extracted on day 17 post- injection and analyzed by flow cytometry. We also examined the effects of combined therapy using the IL-2/αIL-2 complex (IL-2cx) and the CD39 inhibitor POM-1 on tumor progression. In the colon carcinoma (MC38) model, CD39KO mice demonstrated better tumor control ($p \leq 0.05$), higher CD45+ infiltrate ($p \leq 0.05$), and a greater percentage of CD8+ T cells with cytotoxic potential ($p \leq 0.05$) compared to WT mice. In the B16F10-OVA model, CD39KO mice showed similar tumor growth progression but had higher percentages of pre-exhausted (pre-ex) CD8+ T cells ($p \leq 0.05$) than WT mice. Given that pre-ex T cells respond better to immunotherapies, we evaluated the impact of IL-2cx and POM-1 on tumor progression in B16F10-OVA tumor bearing mice. The combination of these therapies significantly improved tumor control compared to monotherapy with IL-2cx or POM-1 ($p \leq 0.05$) and untreated mice ($p \leq 0.01$). This study provides robust evidence that CD39 is a promising therapeutic target for controlling tumor progression, both as a monotherapy and in combination therapies.

472. 244. DOWNREGULATION OF NEOPLASM SUPPRESSOR GENES CHARACTERIZE THE MONOCYTES OF PATIENTS WITH LANGERHANS CELL HISTIOCYTOSIS

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Introduction: Langerhans cell histiocytosis (LCH) is a chronic inflammatory neoplastic disease defined by the invasion of pathological myeloid $CD207^+$ and/or $CD1a^+$ cells into the bone, skin, lung, and lymphoid node. The presentation includes unisystem or multisystem (MS) forms, depending on how many organs are involved. The etiology of LCH is unknown but we have identified that circulating CD14 monocytes of patients with active disease may acquire pathognomonic markers under inflammatory environment. We hypothesized that these monocytes present an altered cell differentiation program promoting genes for survival, migration, proliferation and epithelial-mesenchymal transition. **Aims and Methods**: To analyze the transcriptional program of sorted CD14 monocytes of LCH patients (N=7), an RNAseq was performed with Zymo Research. The profiles were compared with 3 control RNA-seq sets (N= 2 + 3 + 16) and 2 SingleCell- RNA-seq (N = 124 + 190) downloaded from the GEO database. The analysis was conducted using RStudio software. Batch effect correction was implemented utilizing the sva package based on the parametrical empirical Bayes framework, ensuring harmonized combinations. Differential gene expression (DGE) analysis was executed using the DESeq2 package. GSEA analysis was carried out with the log-fold-change values of all transcripts under analysis, employing the clusterProfiler package. **Results:** The DGE analysis showed that the CD14 monocytes from active LCH patients distinctively downregulate key tumor suppressor genes across the 5 data sets used, as the negative regulatory of tyrosine kinase receptors, Sprouty (SPRY), the SAMSN1 negative regulator, Serine/threonine-protein kinase SIK1, and the SAP30 a transcriptional co-repressor of NOTCH signaling. Interestingly, we found upregulated genes for stem cells as the Leucine Rich Repeat Containing G Protein-Coupled Receptor 5 (LGR5) that potentiates the canonical Wnt signaling pathway or the DCLK1 kinase as well as NCAM1.
Conclusion: Our results clearly indicate a differ-

ential transcriptional program in the CD14 monocytes, the precursor of LCH, promoting the release of some neoplasm genes.

473. 246. **ANALYSIS OF NK CELLS IN THE TUMOR INFILTRATE IN PATIENTS WITH BREAST CANCER**

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Background: Adaptive NK (adNK) cells are a subpopulation of CD3-CD56dim NK cells that proliferate following human cytomegalovirus (HCMV) infection. Compared to conventional NK (cNK) cells, aNK cells exhibit enhanced cytokine production via CD16 stimulation (ADCC), increased lifespan, and greater persistence, which highlights their potential for cancer immunotherapy. Recently, we characterized peripheral blood (PB) adNK cells from breast cancer (BC) patients, analyzing both their functional and phenotypic profiles. Additionally, we observed an in vivo expansion of this cell population in patients undergoing HER2-targeted therapy (Bordignon et al 2023). Methodology: We investigated adNK cells within the tumor immune infiltrate using flow cytometry. Our cohort comprised 24 BC patients, from whom we collected PB, tumor, and, in some cases, mammary tissue samples were taken on the day of surgery. We analyzed the adNK cell subset in these samples, defined as LiveCD45+CD3-CD56dimNKG2C+. Results: We observed no significant differences in the proportion of adNK cells across the different tissue types (One way ANOVA). Similar to PB, adNK cells within the tumor infiltrate displayed lower NKp46 expression than their cNK counterparts. Moreover, both adNK and cNK cells showed diminished expression of CD16 and TIGIT within the tumor (Two-way ANOVA with Tukey post-test). Notably, a positive Pearson correlation (ρ=0.7, p=0.02) was observed between the proportion of adNK cells and PD-1+ CD8 T lymphocytes only within the tumor infiltrate. To further characterize these cells, we performed two functional assays where cells were stimulated with PMA and ionomycin. In these assays, adNK cells showed increased degranulation and IFNγ production compared to cNK cells. Conclusions: Our findings suggest that adNK cells may play a role in enhancing the antitumor adaptive immune response. Further research, including additional functional and proliferation assays, is needed to expand our understanding of the biology of these cells in tumor tissues.

474. 247. **TUMOR-INFILTRATING CD4+ T CELLS RECOGNIZE HUMAN RENAL CELL CARCINOMA AND ARE FUNCTIONALLY ORGANIZED INTO CIRCULATING AND TISSUE-RESIDENT COMPARTMENTS**

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Renal cell carcinoma (RCC) is a prevalent and deadly type of kidney cancer that responds to immune checkpoint blockade immunotherapy. However, tumor infiltration of CD8+ T cells have opposite effects on survival. Therefore, conventional CD4+ T (Tconv) cells, including type 1 helper (Th1) and cytotoxic subsets may play a key antitumor role in RCC. To evaluate this, we have performed multiparameter flow cytometry analysis of freshly resected RCC tumors and peripheral blood. Our data indicate that RCC tumors are markedly infiltrated by memory CD4+ T cells with a Th1 profile that recognize autologous RCC cells, but not non- malignant renal tissue, in an HLA-II-dependent manner, as evidenced by upregulation of OX40 and 4-1BB, as well as IFN-γ and TNF-a secretion in coculture assays. In addition, we found that Tconv cells exhibit both tissue-resident and circulating memory phenotypes. The tissue-resident memory subset shows higher levels of PD-1, CD39 and CXCL13 expression, but null or intermediate levels of granzyme B, without expression of perforin and granulysin,

resembling a more exhausted phenotype. In contrast, the circulating memory subset present a cytotoxic subpopulation expressing CX3CR1, perforin, granulysin, high levels of granzyme B and intermediate levels of PD-1. Furthermore, cytotoxic memory CD4+ T cells were enriched in the peripheral blood of RCC patients compared to healthy donors and, hence, it may represent a clonally expanded population. Our data indicate that tumor-infiltrating Tconv cells directly recognize RCC cells and are functionally organized into circulating and tissue-resident compartments with distinct cytotoxic and exhausted profiles, respectively. These findings support the antitumor role and therapeutic potential of CD4+ T cells in human RCC.

475. 268. **TUMOR MICROENVIRONMENT-DRIVEN SUPPRESSION OF DENDRITIC CELL ACTIVATION IN MELANOMA**

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Melanoma presents a growing public health challenge, responsible for 80% of skin cancer deaths. Despite its resistance to conventional treatments, immunotherapy offers a promising alternative by harnessing the immune system to target the tumor. Dendritic cell (DC)-based vaccines offer the potential for generating lasting antitumor responses but are limited by the immunosuppressive phenotype of DCs derived from autologous monocytes of cancer patients used in vaccine development. This study investigates the role of the melanoma tumor microenvironment (TME) in immunomodulation. To evaluate the impact of the TME on DC functionality and phenotype, we developed *in vitro* 3D models, called spheroids, that mimic the melanoma architecture. We constructed homotypic and heterotypic spheroids using various ratios of melanoma cells (B16-OVA) and stromal fibroblasts (NIH/3T3) to replicate melanoma TME complexity closely. Our results demonstrated that fibroblasts conferred structural stability to the spheroids, with higher fibroblast proportions leading to more compact and smaller diameters. We then assessed the activation status of the JAWS II DC line by co-culturing them with these spheroids under basal and LPS-stimulated conditions. Co-culturing DCs with homotypic melanoma spheroids significantly decreased their viability, indicating potential cell death, while co-culturing with homotypic fibroblast spheroids did not affect DC responses to LPS stimulation. Conversely, co-culture with heterotypic spheroids containing different melanoma/fibroblast ratios, inhibited DC activation in response to LPS, indicating the TME's suppressive effect on these immune cells. Considering that melanoma can transform fibroblasts into CAFs with immunosuppressive properties, we first assessed tumor paracrine signaling in the transformation of fibroblasts into CAFs by evaluating proliferation rates and specific CAF markers, finding no significant effects. Ongoing experiments aim to develop genetic engineering strategies to create cell lines to differentiate and isolate coexisting populations within the spheroids. This facilitates a detailed investigation of how direct and indirect communication within the TME contributes to immunosuppression.

476. 269. **DOWN-MODULATION OF MHC-I IN TUMOR CELLS BY BACTERIAL RNA: A POSSIBLE THERAPEUTIC STRATEGY**

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Brucellosis is a zoonotic disease caused by *Brucella* spp bacteria. These pathogens can survive inside macrophages, persisting inside the host. We previously demonstrated that *Brucella abortus* (*Ba*) RNA by a TLR8-dependent mechanism, down-modulates the IFN-gamma-induced MHC-I surface expression on human monocytes/macrophages. Despite not being crucially involved in the response against *Ba*, MHC-I down-modulation may activate Natural Killer (NK) cells. Moreover, NK are key against multiple tumors. Therefore, we aimed to start evaluating the relevance of *Ba* RNA-mediated MHC-I down- modulation in

the context of tumors. For this, we started by stimulating the human glioblastoma cell line (U251), colorectal adenocarcinoma cell line (HT-29), breast cancer cell line (MCF-7) and murine melanoma cell line (B16-OVA) with different doses of *Ba* RNA in the presence of IFN-gamma. MHC-I expression was assessed by flow cytometry. *Ba* RNA diminished the IFN-gamma-induced MHC-I surface expression in U251 ($p<0.001$), HT-29 ($p<0.05$), MCF-7 ($p<0.05$) and B16-OVA ($p<0.05$). *Ba* RNA did not significantly affect cell death as shown by a Zombie violet assay. Then, to corroborate if the receptor was TLR8, U251 cells were stimulated with the hTLR8 agonist ORN06/LyoVec, which could mimic the effect of *Ba* RNA on MHC-I surface expression. Finally, we started to evaluate NK cytotoxicity on tumor cells. For this, U251 cells were loaded with CFSE (10uM) and MHC-I expression was modulated by *Ba* RNA in the presence of IFN-gamma. Cells were then challenged with peripheral blood mononuclear cells (PBMC) for 5h. Preliminary results would indicate that cells treated with *Ba* RNA in the presence of IFN-gamma show a higher percentage of cell death compared to cells treated only with IFN-gamma. Overall, our already established model of MHC-I down- regulation (either by *Ba* RNA or synthetic hTLR8/mTLR7 agonists) could be used as a therapeutic strategy to promote anti-tumor response.

477. 280. MODULATION OF NATURAL KILLER CELL PHENOTYPE AND FUNCTION BY CD29 BLOCKAGE IN MURINE MELANOMA

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Scarce information regarding Natural killer cells (NKs) function within lymph nodes (LNs) have been reported in the context of cancer. Despite their low frequency (0.5-1% of leukocytes), active patrolling of these cells is well characterized with cytolytic activity in peripheral LNs. We previously demonstrated in an immunocompetent syngeneic murine melanoma model that systemic administration of an antibody targeting the integrin CD29 significantly decreased tumor growth. This fact is correlated with increased expression of inflammatory mediators on T-cells of both tumor-infiltrating lymphocytes (TILs) and those residents in the tumor-draining lymph nodes (TdLNs). Since CD29 interacts with CD49b, an integrin expressed by NKs, in this project, we aimed to evaluate whether the systemic administration of anti-CD29 could also modulate the phenotype and function of NKs in the LNs and intratumoral tissue. For this, intraperitoneal administration of anti-CD29 in both tumor-bearing and naïve mice was performed three times per week for a total of 10- 12 days. Then, NKs were characterized in terms of surface receptor expression (CD29, CD49b, CD11b and CD27), perforin/granzyme B content and cytokine production (IFN- gamma and TNF-alpha). Our results indicate that NKs display a distinctive phenotype in the LNs with higher CD11b and IFN-gamma expression compared to NKs from other tissues exclusively in naïve mice. However, the administration of the anti-CD29 did not modify any of the analyzed parameter's on NKs. These results indicate that the anti- tumoral properties of systemic anti-CD29 occurs independently of NKs function.

478. 285. STUDY OF CD4+ TH1, TH2, TH17, TREG CELL SUBPOPULATIONS AND THEIR MAIN CYTOKINES DURING MYELOTOXICITY RELATED TO CHEMOTHERAPY IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia represents the most common type of pediatric cancer. In low- and middle-income countries, the high doses of antineoplastic drugs used in chemotherapy often lead to myelotoxicities, which can increase the risk of mortality. CD4+T cells play a crucial role in coordinating the immune response against cancer cells; it has been described that Th1, Th2, Th17, and Treg cells and their cytokines have an important role in the outcome of the disease and the response to treatment. Therefore, monitoring these cell populations may help assess the presence and severity of myelotoxicities and predict patient survival outcomes or complications. **Objectives:** To determine the changes in the frequency of CD4+ Th1, Th2, Th17, and Treg cells and their cytokine levels during myelotoxicity in induction therapy in pediatric ALL patients. **Methods:** We included pediatric patients with a recent diagnosis of ALL. Blood samples were collected at diagnosis, during, and after the

IT. CD4+ subpopulations were identified by flow cytometry. Myelotoxicities were evaluated using a complete blood count and Common Terminology Criteria. Sociodemographic and clinical variables were analyzed using chi-square or t-test. Cellular population data were assessed with non-parametric tests. A p-value < 0.05 was considered significant. The study the Research and Ethics Committees approved the protocol. **Results:** Patients who completed the IT of chemotherapy with mild and/or moderate myelotoxicities present an increase in the frequency of Th1, Th2, and Th17 cells, with a decrease in Treg. In contrast, patients with severe myelotoxicities show an increase in the percentage of Treg and a reduction in Th1, Th2, and Th17 cells. **Conclusion:** Treg cell population is related to severe myelotoxicities and could represent a therapeutic target.

479. 286. **IN VITRO TARGETING OF TAM RECEPTOR FAMILY REDUCES M2A MACROPHAGE POLARIZATION IN TRIPLE-NEGATIVE BREAST CANCER**

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Background: Breast cancer is the most prevalent and deadly cancer in women worldwide. Triple-negative breast cancer (TNBC) is the most aggressive subtype and has limited treatment options. Tumor-associated macrophages play a critical role in tumor progression and immune evasion. The TAM receptor family (TYRO3, AXL, and MERTK) and its ligands (GAS6 and PROS1) are expressed in cancer cells and are associated with poor prognosis, emerging as potential therapeutic targets. Macrophages also express these receptors, and we hypothesize that inhibiting them will impact macrophage differentiation and enhance antitumor immunity. **Objective:** This study investigates the role of the TAM receptor family in macrophage programming within the TNBC microenvironment and the effects of blocking these pathways. **Methods:** We used flow cytometry to analyze macrophage profile markers CD206, CD163, CD64, and HLA-DR in macrophages cultured from purified CD14+ cells of healthy donors' blood over 7 days. On day 5, we exposed macrophages to 50 % MDA-MB 231 TNBC cell line conditionate media (CM) or vehicle (DMEM). We also tested Annexin V (1 ug/ml) and blocking antibodies against GAS6 and PROS1 to assess TAM receptor involvement. **Results:** TNBC CM significantly increased CD206 expression (CM vs. vehicle, n=10, p=<0.0001) and decreased HLA-DR levels (CM vs. vehicle, n=10, p=0.0285) with no significant changes in CD163 and CD64. Notably, TAM blockade using Annexin V reversed the effect on CD206 expression (n=8, p=0.0033). Preliminary results using anti-GAS6 and anti-PROS1 antibodies together showed similar trends (n=2). **Conclusions:** TNBC cells secrete factors that drive macrophages towards an M2a phenotype, characterized by high CD206 expression associated with anti- inflammatory and tissue repair responses. This process is mediated through interactions between TAM receptors and their ligands. These results suggest that targeting these pathways not only has the potential to directly impact tumor cells but could also enhance the antitumoral immune response.

480. 314. **WNT/ BETA-CATENIN ACTIVATION MODULATES THE PHENOTYPE OF TUMOR-ASSOCIATED MACROPHAGES IN ANAPLASTIC THYROID CANCER**

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Anaplastic Thyroid Cancer (ATC) is heavily infiltrated by tumor-associated macrophages (TAMs). We have reported that treating THP-1 cells (a human monocyte-like cell line) with conditioned media derived from human 8505C ATC cells (ATCCM) induces their activation toward a pro-tumoral phenotype that upregulates β-catenin and associated pathway genes. To investigate the role of the Wnt/β-catenin pathway in the TAM phenotype, a xenograft model of ATC in SCID-NOD mice was used, in which the animals were treated with LGK974 (a Wnt protein secretion inhibitor) or a control. Immunofluorescence assays confirmed β-catenin expression in the tumors. FACS analysis showed a shift toward a more inflammatory profile in TAMs from LGK974-treated mice compared to control- treated ones, with an increased percentage of CD86+ cells and a decreased percentage of CD206+ cells, although no significant differences were observed in iNOS or arginase expression. To evaluate the

effect of Wnt proteins derived from tumor cells or TAM on macrophage cytokine production, THP-1 cells were treated with ATCCM containing or not Wnt proteins in the presence or absence of LGK974. LegendPlex analysis of culture supernatant showed that IL-1β production depends on both macrophages- and tumor cells-derived Wnt proteins while MCP-1 and IL-6 production was independent. To determine if these findings could be observed in patients, we analyzed single-cell RNA sequencing data from biopsies of anaplastic thyroid cancer (ATC) and adjacent normal thyroid tissue, using the framework developed by Lina Lu et al. (GSE193581). Our analysis revealed significant differences in β-catenin and associated pathway genes within the tumor microenvironment of ATC compared to normal adjacent thyroid tissue. Additionally, TAM exhibited an anti-inflammatory profile compared with those from normal thyroid tissue. These results underscore the crucial role of the Wnt/β-catenin pathway in regulating the macrophage phenotype within the tumor microenvironment.

481. 318. **ENDOGENOUS GALECTIN-1 FUELS CD8+ T CELL EXHAUSTION PROGRAMS IN TUMOUR MICROENVIRONMENTS**

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Antitumor immune responses are held in check by a plethora of inhibitory signals that promote tumour-immune escape. Galectin-1 (Gal1), a glycan-binding lectin that recognizes *N*- acetyllactosamine (LacNAc) residues in complex N- and O-glycans causes immunosuppression by triggering apoptosis of activated T cells, inducing tolerogenic dendritic cells and polarizing macrophages towards an M2-like phenotype. Through single-cell RNA sequencing analysis, we found that exhausted $CD8^+$ T cells (Tex) in the tumour microenvironment defined by high PD-1 and TIM-3 expression showed considerably higher levels of Gal1 compared to their effector counterparts, suggesting a potential role of this lectin in modulating $CD8^+$ T cell exhaustion. To test this hypothesis *in vivo,* we used CRISPR-Cas9 on OVA-restricted OT-1 $CD8^+$ T cells to generate Gal1-deficient antigen- specific $CD8^+$ T cells. Silencing was confirmed by Western blot and RT-qPCR. We injected Gal1- sufficient and deficient OT-1 CD8 T cells into B16-OVA-bearing *Rag2*$^{-/-}$ mice. Flow cytometry analysis revealed that Gal1 KO Tex cells displayed an increased cytotoxic phenotype, with elevated expression of Granzyme B ($p<0.05$), IFN-gamma and TNF-alpha ($p<0.01$) compared to WT cells. Additionally, we interrogated *in vitro*-generated Tex (iTex) and observed that Gal-1 KO iTex displayed higher expression of IFN-gamma and Granzyme B. We evaluated whether soluble Gal1 may contribute to T cell exhaustion by interacting with immune checkpoints in a glycan-dependent manner. Using solid-phase binding assays and isothermal titration calorimetry, we demonstrated that Gal1 binds differentially to a set of immune checkpoint receptors, an effect which could be reverted by lactose, suggesting possible autocrine or paracrine effects of the secreted galectin within lymphoid or myeloid cell compartments. Our results suggest that endogenous Gal1 expression in $CD8^+$ Tex cells contributes to their dysfunctional state, possibly through engagement of immune checkpoint receptors. Silencing Gal1 may have therapeutic implications in cancer immunotherapeutic regimens such as immune checkpoint blockade and adoptive cell transfer.

482. 327. **IMPACT OF TRUNCATED O-GLYCANS ON MUC16 SHEDDING AND THEIR ROLE IN CHEMORESISTANCE AND IMMUNOMODULATION IN A MURINE LUNG CANCER MODEL**

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Background: Lung cancer is one of the most commonly diagnosed cancers worldwide and a leading cause of cancer-related mortality. The accumulation of immature O-glycans on cancer cells, like the Tn antigen, is associated with poor prognosis and high metastatic potential. MUC16, a high molecular weight transmembrane mucin, is released from the cell surface following proteolytic cleavage to generate circulating CA125 and a cell surface bound C terminal (CT) fragment. In particular, this fragment has oncogenic properties, influencing tumor chemoresistance. Taking

into account that O-glycosylation regulates extracellular proteolysis, it is interesting to evaluate if truncated O-glycans can favor MUC16 cleavage, MUC16-CT generation and therefore cancer cell aggressive phenotype. **Objectives**: This study aims to analyze if the expression of truncated O-glycans can influence the cleavage of MUC16 and affect tumor cell behavior in a murine lung cancer model. **Methods/Results**: We performed both *in vitro* and *in vivo* studies using a genetically modified murine lung cancer cell line that has altered expression of truncated O-glycans (LL/2 Tn+), in comparison with the parental cell line (LL/2 wt). The *in vitro* experiments confirmed that Tn antigen expression correlated with increased levels of MUC16 expression and CA125 secretion, as well as with an increased chemoresistance to gemcitabine and cisplatin. The *in vivo* assays are currently ongoing, in which we will evaluate serum CA125 expression and tumor expression of MUC16 and other molecules involved in chemoresistance and immunomodulation as p53 and IL-6, among others. We will also analyze the response to treatment with gemcitabine and cisplatin of tumor-bearing mice. **Conclusion**: These findings could provide insights into the role of MUC16 and truncated O-glycans in lung cancer, potentially identifying novel targets for therapeutic intervention.

483. 340. **ROLE OF JERDOSTATIN, A SNAKE-VENOM-DERIVED DISINTEGRIN, IN MODULATING TUMOR ANGIOGENESIS**

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Integrins are a family of transmembrane receptors that mediate cell-cell and cell-extracellular matrix interactions and trigger signaling cascades, which regulate diverse responses including cell adhesion, migration, proliferation, differentiation, and death. Integrin α1β1 is one of the primary collagen-binding receptors expressed in many cell types, including cancer cells and vascular endothelium. There are very few specific inhibitors of the α1β1 receptor, such as Jerdostatin, a peptide derived from snake venom with proven anti-angiogenic properties. However, it has not been studied in cancer angiogenesis models. Our aim is to evaluate the anti-angiogenic potential of Jerdostatin in the context of aberrant glycosylation, in a murine lung cancer model with truncated O-glycosylation and Tn antigen expression, previously developed in our lab. We are currently working on producing recombinant Jerdostatin in insect cells. To achieve this, we have designed a *Drosophila* expression vector (DroExp) with the nucleotide sequence of Jerdostain from *Protobothrops jerdonii*. Our strategy consists in transforming S2 insect cells, followed by the isolation of Jerdostatin using affinity chromatography targeting the Streptactin tag. Subsequently, this tag will be removed by proteolysis with Tobacco Etch Virus protease. Once we obtain the purified protein, we will evaluate its anti-angiogenic potential on a lung cancer murine model with incomplete glycosylation. Firstly, we will assess Jerdostatin capacity to bind Human Umbilical Vein Endothelial Cells. We will further evaluate proliferative, migratory and tube formation capacity on these cells when incubated with Jerdostatin. Finally, if our initial experiments provide promising results, we plan to explore the anti-angiogenic properties of Jerdostatin by injecting mice with murine lung cancer cells exhibiting aberrant glycosylation, which promote highly vascularized tumors. Our investigation will provide a robust model to assess Jerdostatin's therapeutic potential in angiogenesis inhibition. This will shed light on future anti-angiogenic therapies for cancer treatment.

484. 354. **ASSOCIATION OF NKG2D ALLELES WITH NK CELL MARKER EXPRESSION AND COLORECTAL CANCER RISK IN CHILEAN POPULATION**

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Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Natural killer (NK) cells are crucial in anti-tumor immunity, mediated by interactions with tumor cell ligands via the NKG2D receptor. Variants in the NKG2D receptor, such as the rs1049174 SNP, influence receptor activity and expression. The G allele at this SNP is associated with higher NKG2D expression and potentially lower cancer risk due to increased NK cell activity, while the C allele is linked to reduced receptor expression and potentially higher cancer risk. However, these associations have not been studied in the Chilean population. This study aimed to investigate the relevance of the rs1049174 SNP alleles in Chilean population, particularly their association with CRC risk, NKG2D expression, and NK cell-related activity. Blood samples were collected from CRC patients at Hospital Dr. Franco Ravera Zunino and healthy volunteers from the Universidad de Chile. DNA was extracted, and the rs1049174 alleles were analyzed by Sanger sequencing. RNA was extracted to measure mRNA levels of NKG2D, Granzyme B, IFN-gamma, and TNF-alpha by RT-qPCR, correlating NKG2D alleles with these markers. Plasma levels of IFN-gamma and TNF-alpha were also quantified. The study was approved by the Ethics Committee (CEISH) of the Universidad de Chile, and informed consent was obtained from all participants. The results indicated a trend towards lower CRC risk in individuals homozygous for the G allele, who also exhibited higher mRNA levels of NKG2D, Granzyme B, IFN-gamma, and TNF-alpha, alongside elevated plasma levels of the latter two cytokines. These findings suggest that the G allele may confer a protective effect against CRC, potentially through enhanced NK cell-mediated immune responses. Therefore, identifying NKG2D alleles is crucial for understanding their potential role in modulating NK cell activity markers and CRC risk in Chilean population.

485. 388. **EFFECT OF STIMULATORY FACTORS ON MITOCHONDRIAL TRANSFER FROM ORAL SQUAMOUS CELL CARCINOMA CELLS TO CD4+ T LYMPHOCYTES**

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Oral squamous cell carcinoma (OSCC) is the most common neoplasm in the oral cavity and is capable of modulating the anti-tumor response. Mitochondrial transfer has been identified as a form of cell communication, and preliminary data have shown the induction of exhausted CD4+ T lymphocytes through artificial mitochondrial transfer from OSCC cells. However, the factors that trigger natural mitochondrial transfer remain unknown. The aim of this investigation is to evaluate the effect of stimulatory factors on natural mitochondrial transfer from OSCC cells to CD4+ T lymphocytes. For the investigation, a cell line known as HSC-3 was used to represent oral squamous carcinoma cells. CD4+ T lymphocytes were isolated from peripheral blood and then activated for 3 days. The HSC-3 cells were labeled with CellTrace Violet (2.5 mM) and MitoTracker Red (1 µM), while CD4+ T lymphocytes were labeled with an anti-CD4 antibody. HSC-3 cells were stimulated for 14 hours in a 96-well plate with lipopolysaccharide (10 ng/mL), IFN-I (50 ng/mL), TNF-alpha (10 ng/mL), and IL-6 (50 ng/mL) prior to initiating a co-culture system. The ratio of HSC-3 cells to CD4+ T lymphocytes was 10:1. The percentage of mitochondrial transfer was evaluated at 10 minutes, 30 minutes, 1 hour, and 3 hours by flow cytometry. The results showed that the percentage of mitochondrial transfer reached 59.3% at 3 hours under basal conditions, while treated cells exhibited a slightly lower transfer percentage compared to non-stimulated cells at all evaluated times. This study reveals that natural mitochondrial transfer between HSC-3 cells and CD4+ T lymphocytes is independent of the presence of the stimulatory factors studied.

486. 389. **DEVELOPMENT OF AN IN VITRO MODEL TO STUDY THE ABERRANT O-GLYCOSYLATION OF VEGF AND ITS EFFECT ON LUNG CANCER**

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Lung cancer has been the most commonly diagnosed in the past year and the most prevalent in

men and second in women. Malignant cells often have abnormal glycosidic structures contributing to tumor growth and dissemination. The Tn antigen (GalNAc-O-Ser/Thr) is a remarkable example, being expressed in more than 90% of human adenocarcinomas. The main objective of our research is to evaluate whether aberrant glycosylation in lung cancer cells can lead to the presence of truncated glycans in VEGF-A produced by Tn+ tumor cells, and how these modifications may affect the interaction of VEGF-A with its receptor VEGFR2, thereby modulating VEGF-A pro-angiogenic function. Our research group has generated a preclinical lung cancer model with aberrant glycosylation, expressing the Tn antigen from the Lewis lung carcinoma murine line, LL/2. We evaluated the expression and production of VEGF-A in LL/2 wt and Tn+ cells using quantitative real-time PCR and an ELISA assay, respectively. We also analyzed the glyco-phenotype of both cell lines by flow cytometry. Subsequently, we performed transfection of LL/2 wt and Tn+ cells with lentiviral particles to transduce and overexpress VEGF-A. Firstly, we confirmed Tn antigen expression in LL/2 Tn+ cells using biotinylated lectins by flow cytometry. Moreover, Tn+ cells produce larger and more vascularized tumors than the parental line, due to the secretion of VEGF-A that induces increased migration and tubulogenesis by endothelial cells, thereby promoting tumor angiogenesis. We also observed that LL/2 Tn+ cells express higher levels of VEGF-A mRNA and protein compared to LL/2 wt cells. Finally, we generated LL/2 wt and Tn+ cells that overexpress VEGF-A by lentiviral transduction.

In conclusion, the development of this model will allow us to evaluate the effect of truncated O-glycosylation on VEGF-A signaling and function. In upcoming work, VEGF-A wt and Tn+ will be purified for functional assays.

487. 404. **MICROSATELLITE INSTABILITY AND MLH1 METHYLATION AS PROGNOSTIC MARKERS IN LOW-AGGRESSIVENESS PROSTATE CANCER**

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Background: Prostate tumors display significant variability in aggressiveness, which is assessed using the Gleason score. Despite advancements in diagnostics, differences in tumor aggressiveness and patient responses to conventional treatments remain unclear, emphasizing the need for personalized approaches. Microsatellite instability (MSI), resulting from DNA repair defects, contributes to tumor aggressiveness and is a crucial marker for identifying candidates for immunotherapy. Additionally, MLH1 promoter methylation has been linked to gene silencing and can serve as a significant prognostic factor in prostate cancer (PCa) by affecting DNA mismatch repair. Objective: To assess the correlation between MSI and Gleason scores in patients with PCa and MLH1 methylation in MSI cases. Methods: The study involved 35 patients with histopathologically confirmed PCa. Formalin-fixed, paraffin-embedded tumor tissues were processed for DNA extraction. MSI analysis was conducted using multiplex PCR with five markers: NR-27, NR-21, NR-24, BAT-25, and BAT-26 (Type-it Microsatellite PCR kit, Qiagen). Fragment analysis was performed via capillary electrophoresis using the SeqStudio Genetic Analyzer (Applied Biosystems), and the data was interpreted using Microsatellite Analysis Thermo Fisher Connect™ software. MLH1 methylation was assessed by bisufite sequencing followed by MS-PCR. Results: MSI was detected in 9% of the study cohort, with 6% showing low MSI (one altered marker) and 3% showing high MSI (two or more altered markers). All MSI-positive samples had Gleason scores of 6 or 7. Interestingly, MSI was present in less aggressive tumors, with a frequency of 9%, higher than reported in the literature. Additionally, MLH1 promoter methylation was assessed in MSI-positive samples, revealing two positive cases. These cases exhibited lower PSA levels compared to non-methylated cases. Conclusion: The study highlights the presence of MSI in less aggressive prostate tumors, with a higher incidence than previously documented. MLH1 promoter methylation may be linked to lower PSA levels, suggesting potential implications for prognosis and treatment stratification.

488. 417. **EXPLORING IMMUNE INFILTRATION IN BREAST ADIPOSE TISSUE: IMPACT OF TUMOR PROXIMITY ON IMMUNE CELL IDENTITY**

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The microenvironment, mainly adipose tissue, play a key role in breast cancer progression, with the immune response balancing between cancer suppression and promotion. This study aimed to analyze immune components in human breast adipose stromal explants to explore differences between physiological and tumor contexts. We performed a histological analysis of breast adipose explants obtained from patients with breast cancer, including those from areas: adjacent to the tumor (ADJ, n = 29) and >2 cm away (DIST, n = 27), alongside normal controls (Normal, n = 24). The samples were stained with hematoxylin and eosin to assess immune infiltration. Immune infiltrate was detected in 45.8% of Normal, 51.7% of ADJ and 48.1% of DIST explants. Infiltration levels were categorized as – (negative) or +, ++ and +++ (positive, with each category corresponding to the average density of the infiltrate). Combining the ++ and +++ categories, 12.5% of Normal and 11.1% of DIST fell into this group, whereas 17.2% of ADJ explants showed higher infiltration, with ADJ being the only group with +++ samples. We identified foci of lymphocytes, macrophages, mast cells, basophils, eosinophils and neutrophils in ADJ and DIST explants. Normal explants had a significant presence of neutrophils (9.1%) and eosinophils were prominent in the ADJ (20.0%) and DIST (15.4%) explants. Interestingly, in the samples with a predominance of adipose tissue, 50% of the Normal explants with infiltration showed a higher lymphocytic infiltrate, compared to 86.7% in ADJ and 81.8% in DIST. Similarly, 50% of the Normal explants showed a higher frequency of mast cells, while only 33.3% of ADJ and 18.2% of DIST did. Notably, crown-like structures were absent in all explants. These findings suggest that leukocyte proportions and identities in the breast microenvironment vary depending on the tumor-associated or healthy contexts, with adipocytes potentially influencing leukocyte infiltration through secreted signals.

489.419. **COULD THE DIFFERENCES BETWEEN TWO COHORTS OF THE SAME DISCONTINUATION TRIAL HAVE BEEN INFLUENCED BY THE COVID-19 PANDEMIC?**

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Background: Treatment-free remission (TFR) is a key therapeutic goal for chronic myeloid leukemia patients in deep molecular response. While predicting patient outcome remains challenging, NK cells seem crucial. We conducted an immunological sub-study from the Argentina Stop Trial (AST), including 46 patients in 2019 (AST1), and 35 new patients between 2022 and 2023 (AST2). Objectives: To characterize NK cell subsets in patients attempting TFR. Methods: Peripheral blood mononuclear cell samples were collected before stopping treatment. Phenotype was assessed by flow cytometry (BD FACS Canto™II), and the remaining cells were cultured with K562 cell line, to measure degranulation and IFNγ production. Data were analyzed using FlowJo. For statistical analysis, Mann Whitney test was performed to compare variables between groups ($p<0.05$ was considered statistically significant), using GraphPad Prism. Results: Non-relapsing patients from AST1 exhibited NK subpopulations with cytomegalovirus-related memory features, high expression of cytotoxicity markers, and robust functionality (degranulation and IFNγ production). In contrast, in AST2 we were unable to report an association with clinical outcome. Remarkably, though clinical variables were similar, we observed significant differences between cohorts, regarding several immunological parameters. NK cell percentages, CD16 and CD57 were significantly reduced in AST2 (p=0,0051; p=0,0222; p=0,0033, respectively), whereas NKp46, NKp44 and PD-1 levels were significantly increased (p=0,0081; p<0,0001; p<0,0001, respectively). AST2 demonstrated higher global functional-

ity and more memory-like subpopulations with enhanced functional performance (p<0.0001 in most cases). Given the enrollment time of both cohorts and that they appear to be clinically homogeneous, we consider that COVID could be impacting the immune landscape. Conclusion: The influence of the COVID pandemic and the different vaccine platforms on NK cells cannot be underestimated when evaluating immunological populations. At this time, as we were unable to correlate these findings with patient outcome, we are collecting complementary information to help us establish stronger associations with COVID infection.

490. 443. **SPATIO-TEMPORAL LANDSCAPE OF MACROPHAGE-LYMPHOCYTE INTERACTION IN COLORECTAL CANCER**

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Background: Colorectal cancer (CRC) has a high mortality rate and has significantly increased in incidence among younger people lately. The role of macrophages in CRC is controversial due to the lack of accurate markers able to define their density, spatial distribution, and interaction with lymphocytes. **Methods:** Using multiparametric flow cytometry (FC) we investigated macrophage subsets in human CRC samples and murine colitis-associated CRC model. By multispectral immunohistochemistry (mIF), we analyzed the macrophage landscape and their respective interactions with CD3+ T lymphocytes in 61 CRC patients' histological slides in four distinct zones: non-tumor tissue (NTT), stroma (ST), tumor margin (TM), and tumor center (TC). **Results:** By FC, we noted an elevated proportion of CADM1+-TREM2+ TAMs in tumors compared to paired non-tumor tissues, while FOLR2+ TRM frequency shows the opposite trend. Using mIF, we found a significant increase of CD14+FOLR2+ and FOLR2+ subsets in NTT and ST, while TREM2+ and CD14+ cells were increased at TM and TC. Importantly, CD14+, FOLR2+, CD14+FOLR2+ and CD3+ cells were significantly higher at TC in patients free of lymph node tumoral invasion. Interestingly, higher FOLR2+ Macrophage-T cell interactions were noted at NTT compared to ST, even though ST presented higher macrophages density. Furthermore, all macrophage subsets similarly interact with T lymphocytes at TM, irrespectively of phenotype. **Conclusions:** Our data suggest specific macrophage subsets interact with lymphocytes in a spatially dependent manner, but their impact on patients' survival remains undetermined.

491. 444. **CONTRASTING ROLES OF THE PROTOTYPES GALECTIN-2 AND GALECTIN-1 IN GLIOBLASTOMA**

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Aberrant glycosylation is a hallmark of malignant transformation, influencing immune responses, tumor growth, and invasiveness. Galectins, a family of glycan-binding proteins, play crucial roles in these processes. This study investigates the con-

trasting roles of Galectin-2 (Gal-2) and Galectin-1 (Gal-1) in glioblastoma (GBM), focusing on their effects on tumor progression and the tumor microenvironment. While Gal-1 is known for its protumorigenic functions and their high expression which correlate with poor clinical outcomes in cancer patients, our bioinformatic analyses revealed that in melanoma and colorectal cancer, higher Gal-2 expression correlates with increased immune cell infiltration, notably M1-type macrophages, and improved patient overall survival. Experimentally, we found that Gal-2-deficient mice exhibited larger tumor volumes and worse outcomes in melanoma and colorectal cancer models, suggesting a protective role for Gal-2. Given GBM's poor prognosis and the abundance of macrophages and microglia in its tumor infiltrates, we explored Gal-2's role in this aggressive cancer. We found that four patient-derived GBM cell lines (PDCs) exhibited significantly higher Gal-1 expression compared to non-tumor neuroprogenitors, while Gal-2 levels were similar. Upon exposure of PDCs to BMP4, a morphogen with GBM-differentiating capacity, Gal-1 expression decreased, and Gal-2 expression increased, indicating that Gal-2 may be linked to a less aggressive cellular phenotype. Furthermore, a comparison of Gal-1 and Gal-2 expression between PDCs and their corresponding biopsies showed an opposite pattern: Gal-1 was higher in PDCs, while Gal-2 was elevated in tumor biopsies. Finally, in an immunocompetent mouse model of GBM, Gal-2 deficiency was associated with decreased survival ($p<0.05$), underscoring Gal-2's potential role as a key regulator of the GBM tumor microenvironment. In conclusion, our findings highlight the opposing roles of Gal-2 and Gal-1 in GBM progression and suggest that Gal-2-based agents could serve as a promising therapeutic strategies for GBM treatment.

492. 468. **RNASEQ DECONVOLUTION ALGORITHMS HIGHLIGHT THE IMPORTANCE OF NK CELLS IN RESISTANCE TO NEOADJUVANT CHEMOTHERAPY IN BREAST CANCER**

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Background: Breast cancer is a heterogeneous and complex disease with significant individual differences in molecular immunophenotype, biological behavior, histopathological morphology, and response to chemotherapy. The presence of tumor-infiltrating lymphocytes in breast cancer has gained considerable attention due to growing evidence of their role in therapy response, particularly in the response to neoadjuvant chemotherapy. **Objective:** This study aimed to evaluated tumor infiltration by immune cells using next-generation sequencing and immune cell deconvolution algorithms. **Methods:** Samples from twenty-one breast cancer patients who received neoadjuvant chemotherapy were classified as either sensitive or resistant according to their residual cancer burden score; nine were classified as sensitive and twelve as resistant. Biopsies from all patients were taken before chemotherapy treatment, and total RNA sequencing (RNA-seq) was individually performed. The sequencing quality was assessed, and alignment algorithms were used to generate tables of transcripts abundance. Immune cell infiltration was determined in all samples using deconvolution algorithms. This was achieved with the IMMUNEDECONV package on the R platform, utilizing the Quantiseq, xCell, and Epic algorithms. **Results:** The Epic and Quantiseq algorithms showed that an NK cell-rich infiltrate was significantly associated with a good response to neoadjuvant chemotherapy ($p=0.03$ and $p=0.012$ respectively). These results were consistent with our previous findings that NK cell markers are more expressed in responsive patients. The xCell algorithm indicated significant differences in the presence of granulocyte-monocyte progenitors, mast cells, CD4 Th1 cells, CD4 Th2 cells, and Tregs. While NK cells also tended to be more abundant in responders, the association was not statistically significant ($p=0.16$). **Conclusion:**Immune cell infiltration rich in NK cells is an important characteristic associated with the response to neoadjuvant chemotherapy, as shown by deconvolution algorithms for bulk RNA-seq. Further experiments using immunohistochemistry and specific immune cell markers are needed to confirm these findings.

493. 525. **EXPRESSION AND KNOCK OUT GENERATION OF HISTONE 1.3 IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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Background: Histones 1 (H1) play a key role in transcription regulation through epigenetic modulation of chromatin conformation. Histone 1.3 (H1.3) is upregulated in ovarian and pancreatic cancer. Chronic lymphocytic leukemia (CLL) is a B cell neoplasia in which disease progression associates to chromatin activation. We previously described that the mutagenic enzyme AID introduces loss-of-function mutations in H1.3 and enhances disease aggressiveness. Objectives: We aim to study the expression profile and the role of H1.3 in CLL. We hypothesize that H1.3 expression levels or mutant variants impact the phenotype and function of CLL cells. Methods: We studied H1.3 gene expression in CLL by analyzing available databases, we evaluated the protein level of H1.3 in highly purified CLL cells from primary patient samples and we generated H1.3 stable knock outs (KO) clones by CRISPR/Cas9 technology and the limiting dilution technique on the cell line MEC-1. Results: Our analysis on gene databases showed no differences in H1.3 expression between normal B cells and CLL neoplasic cells (n=8, $p>0.05$) or when comparing poor versus good prognostic groups of patients (n=58, $p>0.05$). We detected a downregulation of H1.3 in CLL cells activated with CD40L or activated by T cells (n=5, $p<0.05$). Preliminary results of H1.3 Western Blots performed in our lab on CLL cases from different prognosis groups and in resting and activated conditions go in line with the gene databases analysis. Three H1.3 stable KO MEC-1 clones were obtained. Excision of the targeted gDNA was verified by end point PCR and lack of H1.3 expression was confirmed by WB. MEC-1 H1.3 KO cells showed increased total RNA synthesis levels and upregulated CD86 expression (n=6, $p<0.05$). Conclusion: We conclude that H1.3 expression is linked to CLL cell activation and have generated a new KO tool for the study of this nuclear protein in B-cell leukemia.

494. 538. LYMPHODEPLETING PRECONDITIONING IMPAIRS LONG-TERM HOST ANTITUMOR T CELL IMMUNITY INDUCED BY ADOPTIVE CELL THERAPY

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Adoptive cell therapy (ACT) using tumor-specific cytotoxic T cells (CTLs) has shown great efficacy in hematological cancers, but remains ineffective against solid tumors and often generates resistance through the emergence of cancer cells that lose expression of targeted antigens. Lymphodepleting preconditioning with cyclophosphamide prior to ACT is used to support engraftment of transferred CTLs. However, its effect on long-term ACT-mediated antitumor protection has not been established. To study this, B16F10-OTI tumor-bearing mice were intravenously transferred with *in vitro* activated OTI $CD8^+$ T cells and some mice received two intraperitoneal doses of cyclophosphamide prior to ACT. Here, we observed that adoptive transfer of TCR-transgenic $CD8^+$ T cells eliminates established murine melanoma tumors, with concomitant expansion of host $CD8^+$ T cells exhibiting tumor-reactive phenotypes, through mechanism dependent on type 1 conventional dendritic cells (cDC1). Furthermore, host $CD8^+$ T cells rejected a rechallenge with ACT-resistant melanoma cells lacking the targeted antigen. Cyclophosphamide preconditioning expanded transferred $CD8^+$ T cells and promoted the rejection of primary tumors, but resulted in long-lasting depletion of endogenous $CD8^+$ T cells and cDC1 in tumors and lymph nodes. As a result, cyclophosphamide-mediated lymphodepletion impaired host antitumor immunity and long-term protection against rechallenge. These

results demonstrate the dual role of lymphodepleting preconditioning in ACT by supporting expansion of transferred cells and rejection of primary tumors, but impairing long-term antitumor immunity, which may ultimately favor resistance to ACT.

495. 542. HETEROGENEOUS TISSUE-RESIDENT MEMORY CD8 T CELLS ARE ESTABLISHED IN BREAST CANCER TUMORS AND INVADED LYMPH NODES

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Tissue-resident memory CD8+ T (Trm) cells are pivotal mediators of the antitumor immune response in breast cancer (BC). Trm cells infiltrating BC tumors associates with improved survival and response to immunotherapy. However, the role of Trm cells in metastatic spreading has not been established yet. We characterized Trm cells in both primary tumors and metastatic lymph nodes (mLN) from BC patients using multiparametric flow cytometry. We found that Trm cells are a highly represented population within the memory compartment in both primary tumors and mLN, but they are absent in non-invaded LNs. These Trm cells express other residency markers, like CD49a, higher levels of activation markers, such as CD27 and 4-1BB, and tumor-reactive markers CD39 and PD-1, as compared to circulating CD8+ T cells. Interestingly, the frequency of tumor Trm cells correlate with activated type I conventional dendritic cells (cDC1) in mLNs and tumors. Furthermore, single-cell RNA and TCR sequencing revealed that Trm cells are a highly heterogeneous population, encompassing progenitor, activated, exhausted and type 17-like cell states. Trm cells from primary tumors and mLN share multiple TCRs, suggesting a common clonal origin. These results indicate that Trm cells are key mediators of the antitumor immunity in BC tumors and mLN.

496. 546. UNVEILING THE ROLE OF MDSCS IN PROSTATE CANCER: INSIGHTS INTO IMMUNE EVASION MECHANISMS

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Background: Myeloid-derived suppressor cells (MDSCs) play a crucial role in immune regulation, particularly in cancer. Their immunosuppressive function impairs effector cytotoxic cells, aiding cancer cell immune evasion. Elevated MDSC levels are observed in cancer patients, including those with prostate cancer, potentially contributing to tumor progression. However, the mechanisms of MDSC immunosuppression are not well understood. Objectives: This study evaluates circulating MDSC levels and the expression of immunoregulatory molecules (CD73, GAL9, PD-L1, SIRPα, CD47) in prostate cancer patients compared to healthy subjects. Methods: Conducted at the Western Biomedical Research Center, this prospective cohort study involved blood samples from healthy subjects (n=4) and prostate cancer patients (n=10). Peripheral blood mononuclear cells (PBMCs) were isolated and stained with antibodies for CD11b, CD14, CD15, CD33, HLA-DR, lineage markers (CD3, CD19, CD20, CD56), CD73, GAL9, PD-L1, SIRPα, and CD47. Flow cytometry was used for analysis, with data processed using FlowJo software. MDSC subpopulations were defined per Cassetta et al. (2020). Statistical analysis was performed using Student's t-test. Results: Prostate cancer patients showed significantly elevated circulating MDSCs, primarily due to increased PMN-MDSCs. M-MDSCs and e-MDSCs did not show significant

differences. Notably, the e- MDSC subpopulation demonstrated significant overexpression of CD73, GAL9, PD-L1, and CD47. The PMN-MDSC subpopulation showed significant overexpression of CD73 and CD47. Additionally, the M- MDSC subpopulation exhibited significant overexpression of CD47. Conclusion: MDSCs are elevated in prostate cancer patients, consistent with previous studies, highlighting their role in the disease. MDSCs from these patients exhibit elevated levels of CD73, GAL9, PD-L1, and CD47. The novel observation of CD47 overexpression across all MDSC subpopulations underscores its role in immune evasion by inhibiting tumor cell phagocytosis. These findings enhance our understanding of MDSC-mediated immunosuppression and may inform future therapeutic strategies.

497. 557. **PIRFENIDONE ENHANCES TUMOR IMMUNOSURVEILLANCE IN EXPERIMENTAL HEPATOCARCINOMA BY RESTRICTING THE INDUCTION OF REGULATORY T CELLS THROUGH THE CANONICAL TGF-BETA PATHWAY**

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Introduction: Hepatocellular carcinoma (HCC), the most common liver cancer, often arises from chronic liver fibrosis and inflammation, which creates a tumor-friendly environment with weakened immune responses and disrupted TGF-β pathways. Since TGF-β is linked to HCC progression, targeting it could be a preventive strategy. Pirfenidone (PFD), which reverses liver cirrhosis, shows promise as a treatment, but its effectiveness for HCC is still being studied. Objective: To assess PFD's therapeutic effects on the tumor and immune environment in experimental hepatocellular carcinoma. Methodology: Fischer-344 rats were divided into three groups: control (CTL), HCC (damage), and HCC treated with pirfenidone (HCC/PFD). The HCC groups received weekly diethylnitrosamine (DEN) and 2- acetylaminofluorene (2AAF), with the treatment group also receiving daily PFD. Tissues were then collected for molecular analysis of immune, fibrotic, and malignancy markers. Results: PFD treatment reduced the size and incidence of cancerous nodules by 58.94%. It also inhibited hepatic stellate cell activation, as shown by reduced α-SMA levels and decreased collagen I and III accumulation, improving liver structure and reducing fibrotic bridges. PFD lowered the number of initiated cells and oncoproteins (GPC-3 and Ki-67) while boosting pro-apoptotic proteins like p53. Additionally, PFD shifted the immune environment by decreasing IL-10, IL-17, and IL-1β levels, restoring IL-6, and enhancing CD161+ lymphoid cell migration into tumors. It also limited the TGF- β pathway by preventing SMAD2 and SMAD3 nuclear translocation, reducing regulatory T cell polarization, and improving immune control over tumors. Conclusion: PFD notably slowed fibrosis and cancer development by improving the tumor's hypoxic and metabolic profile, boosting immune surveillance, and inhibiting pro-tumoral responses. This suggests PFD could enhance survival for HCC patients and serve as a preventative option for those at high risk of developing liver cancer.

498. 560. **LONG-TERM EFFICACY OF ADOPTIVE CELL THERAPY IS DETERMINED BY HOST CD8+ T CELLS AND UNDERMINED BY LYMPHODEPLETING PRECONDITIONING**

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Adoptive T cell therapy (ACT) has demonstrated remarkable e]icacy in treating hematological cancers. However, its e]icacy against solid tumors remains limited and the emergence of cancer cells that lose expression of targeted antigens

often promotes resistance to ACT. Importantly, the mechanisms underlying e]ective and durable ACT- mediated tumor control are incompletely understood. Here, we show that adoptive transfer of TCR-transgenic CD8+ T cells eliminates established murine melanoma tumors, with concomitant accumulation of tumor-infiltrating CD8+ T cells exhibiting both progenitor-exhausted and terminally-di]erentiated phenotypes. Interestingly, host CD8+ T cells contributed to ACT-mediated elimination of primary tumors and rejected ACT-resistant melanoma cells lacking the targeted antigen. Mechanistically, ACT induced TNF-α- and cross-presenting dendritic cell-dependent tumor accumulation of endogenous CD8+ T cells and e]ective tumor elimination. Importantly, although lymphodepleting preconditioning enhanced ACT-mediated tumor elimination, it abrogated host antitumor immunity and protection against ACT- resistant melanoma cells. Enrichment of transcriptional signatures associated with TNF-α signaling, cross-presenting dendritic cells and tumor-specific CD8+ T cells in human melanoma tumors correlated with favorable responses to ACT and increased survival. Our findings reveal that long-term e]icacy of ACT is determined by the interplay between transferred and endogenous CD8+ T cells and is undermined by lymphodepleting preconditioning, which ultimately favors ACT resistance.

499. 577. **INFLUENCE OF THE CHOLINERGIC SYSTEM IN THE PATHOGENESIS OF GLIOBLASTOMA MULTIFORME**

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Glioblastoma multiforme (GBM) is the most aggressive brain tumor. Acetylcholine (ACh) is a neurotransmitter that modulates cell survival in immune cells and tumors. This study aimed to evaluate the role of the cholinergic system (CS) in GBM. We analyzed the expression and function of ACh receptors in GBM datasets and found that increased expression of M3 cholinergic muscarinic receptors (CHRM3). It was associated with decreased survival in GBM patients. This finding was confirmed in human GBM tumor biopsies (GBM-b), which also showed CHRM3 expression. Additionally, we investigated the role of thymic stromal lymphopoietin (TSLP), previously linked to GBM pathogenesis, and observed that TSLP increased CHRM3 expression in the U251 cell line (*$p < 0.05$). We evaluated the effects of ACh and TSLP on apoptosis in the U251 cell line using a 3D model with Temozolomide (TMZ). TMZ treatment induced cell death, but ACh enhanced this effect, while TSLP prevented TMZ-induced apoptosis (*$p < 0.05$). We also assessed the impact of CS and TSLP on U251 cell line spheroids, noting an increase in diameter with both CS agonists and TSLP (*$p < 0.05$, **$p < 0.005$). TSLP treatment led to increased proliferation in spheroids, while CS agonists did not. vascular endothelial growth factor (VEGF) production was evaluated in the U251 cell line, showing a significant increase in VEGF production with Carbacol treatment in both 3D and 2D models (**$p < 0.005$). TSLP also elevated VEGF and PDL-1 levels. These observations let as to conclude that probably TH2 profile agonists such as TSLP and ACh seek a connection that influences the pathogenesis of GBM.

500. 585. **TUMOR-INFILTRATING REGULATORY T CELLS ARE THYMUS-DERIVED**

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The presence of FoxP3 regulatory T cells (Treg) in the tumor microenvironment has been associated with poor clinical outcomes. However, the nature of the infiltrating Tregs, particularly whether they represent thymic-derived Tregs or CD4 T cells that undergo a Treg differentiation process locally, remains an open question. The aim of this work was to determine whether CD4 T cells specific to a tumor-derived antigen become FoxP3+ Tregs in the tumor microenvironment or in tumor draining lymph nodes. B16 murine melanoma cells that expressed ovalbumin (B16-OVA) or not (B16) were inoculated into CD45.1+ wild-type

B6 mice. Naïve CD4+ CD45.2+ Foxp3^{GFP} OT-II cells (specific for OVA) were adoptively transferred. CD4 T cell migration into the tumor and tumor draining lymph nodes was analyzed. FoxP3 expression in transferred OVA- specific cells was quantified. Migration of adoptively transferred OT-II cells into the tumor was negligible, disregarding whether the tumor expressed OVA or not. Virtually all FoxP3 CD4 T cells found in the tumor represented endogenous Helios+ thymic-derived Tregs. OT-II cells recovered from tumor draining lymph nodes were mostly FoxP3 negative. These results were not affected by the capacity of the mouse or the tumor to produce TGF-beta. These results suggest that the regulatory T cells that infiltrate murine melanoma are thymic-derived and that local differentiation of FoxP3+ regulatory T cells from naïve CD4 T cells is negligible, even when CD4 T cells express a TCR able to recognize a tumor-derived antigen with high affinity.

501. 595. **HELIOS LIMITS CD8 T CELL ANTITUMOR CAPACITY**

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Helios (*Ikzf2*) is a transcription factor associated with suppressive function in thymic- derived CD4 regulatory T cells. Previously, we documented Helios expression in CD8 cells exposed to antigen presented as self or associated with malignant cells. Here, we analyzed whether Helios is induced in tumor infiltrating CD8 T cells and determined the effects of its absence. In mice with B16 melanoma, Helios upregulation was observed in tumor infiltrating CD8 T cells. Cells from tumor draining lymph nodes remained Helios negative. Helios was induced by antigen activation, as it was not observed in OT-I cells from tumors devoid of ovalbumin. Helios expression was documented in CD8 T cells infiltrating tumors from patients with colon cancer. We found that the exhaustion-associated transcription factor TOX was co-expressed with Helios in most cells. Helios deficiency in T cells (Cd4-Cre) and in CD8 T cells (E8III-Cre) decreased the growth of melanoma and colon adenocarcinoma in a mouse model. This was associated with decreased abundance of terminally exhausted CD8 T cells and increased frequency of TCF-1^{+} cells. We generated *Pdcd1* and *Ikzf2* double KO mice (dKO). dKO mice, controlled tumor growth better than mice with isolated deficiencies. Tumors did not grow in a large fraction of dKO mice. We generated a Helios-reporter cell line to screen for small molecules capable of inhibiting Helios expression. We validated the effect of the top hits in primary CD8 T cells and found one compound that improved anti-tumor capacity of CD8 T cells *in vivo*. These results indicate that Helios is induced in CD8 T cells by the tumor microenvironment and that its presence curbs the anti-tumor capacity of CD8 T cells. We have discovered a novel inhibitor of Helios that improves the anti-tumor response in a murine system.

502. 612. **TRIMETHYLGLYCINE RESTORES SENSITIVITY TO 5-FLUOROURACIL THROUGH DECREASED EXPRESSION OF THYMIDYLATE SYNTHASE AND TNFALPHA IN COLORECTAL CANCER TUMORAL CELLS**

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Intrinsic or acquired resistance to chemotherapeutic drugs is the main cause of failure in therapy regimens, leading to relapse and death in colorectal cancer (CRC) patients. The chemotherapeutic agent 5-Fluorouracil (5-FU) remains a mainstay in therapeutic combinations. Unfortunately, side effects and chemoresistance to these drugs are common problems. Thus, finding new strategies and alternatives to enhance efficacy or overcome chemoresistance induced by conventional chemotherapies has become necessary. In previous work, we evaluated the potential use of

Trimethylglycine (TMG), a natural compound with anti-inflammatory properties, as a possible adjuvant to 5-FU in an in vivo model of colitis-associated colon cancer (CAC). These results suggest that TMG could act by regulating genes associated with chemoresistance. However, the regulatory mechanism of TMG is not yet fully understood. To address this, we generated a chemoresistant human colorectal cancer cell line (HCT-116R) and a mouse model with CT26 cells to induce tumor implantation. Our goal was to determine the mechanism by which TMG influences sensitivity to 5-FU. We found that TMG negatively regulates thymidylate synthase, one of the most important molecules related to resistance to 5-FU, thus restoring drug sensitivity and inducing apoptosis in tumor cells. Additionally, TMG treatment in combination with 5-FU significantly reduces the proinflammatory cytokines TNF-α and IL-17a. Assays with HCT-116 cells demonstrated that TMG effectively inhibits cell migration induced by TNF-α. Our data identify TMG as a valuable adjuvant therapy in colon cancer, as sensitizer of tumor cells by regulating enzymes that metabolize 5-FU and proinflammatory cytokines associated with malignancy

503. 616. **TUMOR-SPECIFIC MEMORY CD8+ T CELLS INFILTRATE RENAL CELL CARCINOMA TUMORS AS CIRCULATING PROGENITORS THAT DIFFERENTIATE INTO TISSUE-RESIDENT SUBSETS AND UNDERGO EXHAUSTION**

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Renal cell carcinoma (RCC) is a prevalent and deadly type of kidney cancer that responds to immune checkpoint blockade immunotherapy. However, tumor infiltration of CD8+ T cells has contrasting effects on patient survival, which may be explained by a high phenotypic and functional heterogeneity. In this study we showed that RCC tumors are infiltrated by circulating (Tcirc) and tissue-resident (Trm) memory $CD8^+$ T cells. Trm cells ($CD69^+CD103^+$) and $CD69^+CD103^-$ memory populations displayed higher HLA-dependent reactivity against autologous RCC cells than Tcirc ($CD69^-CD103^-$) cells. None of these subsets recognized non- malignant renal tissue. Transcriptomics analysis showed that both Trm and $CD69^+CD103^-$ memory sub-populations exhibit a tissue-residency program, with the latter displaying a higher exhausted phenotype. Single-cell transcriptomics revealed a rather heterogenous composition of memory $CD8^+$ T cells, including cytotoxic effector-memory and Tcm-like progenitor circulating subsets, as well as multiple Trm subsets, including progenitor, activated, stressed and exhausted states. Notably, our analysis revealed previously unappreciated cell subsets, including regulatory and type-17 memory $CD8^+$ T cells. Furthermore, clonally expanded T cell receptors (TCRs) were preferentially found in activated and exhausted Trm cells and shared across several subsets. TCR and trajectory analyses indicate that circulating Tcm-like precursors rapidly lose their circulating and progenitor transcriptional programs within the tumor, followed by the activation and acquisition of a tissue-resident differentiation, which either become quiescent progenitors or progressively acquire terminally differentiated and exhausted phenotypes. Overall, our study suggests that human memory $CD8^+$ T cells infiltrating RCC tumors originate from Tcirc progenitors that differentiate into Trm cells that undergo progressive exhaustion. Our findings have clear implications for clinical translation, particularly for adoptive cell therapy.

504. 629. **ADENOSINE A2A RECEPTOR: A KEY TARGET FOR OVERCOMING IMMUNE**

EVASION AND TUMOR AGGRESSIVENESS

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Abstract: The cancer-immune cycle is crucial in the progression of cancer, with a key step occurring within the tumor microenvironment that involves immune checkpoints. While immunotherapy has achieved remarkable success in treating cancers such as melanoma, a substantial number of patients and various other cancer types, including breast carcinoma and glioma, still exhibit resistance to these treatments. Indeed, preclinical and clinical studies have revealed compensatory feedback mechanisms linked to the blockade of checkpoints. The immune checkpoint, Adenosine A2A Receptor (A2AR) stands out as a promising target due to its involvement in key processes such as hypoxia, invasion, angiogenesis, metastasis, and chemoresistance. Here we aimed at determining the clinical and prognostic significance of the A2AR. Transcript and protein analysis revealed a significant overexpression of A2AR in cancers like breast and gliomas, associated with aggressive clinical features and reduced patient survival, suggesting that this receptor could serve as a predictive biomarker for tumor aggressiveness and patient survival. Furthermore, A2AR expression in tumor-infiltrating immune cells was closely correlated with the expression of PD1 and CTLA4. *In silico* analyses highlighted increased infiltration of pro-tumoral cells in $A2AR^{high}$ tumors, and CD8+ T cells from this group exhibited an exhausted functional profile. GSEA analysis indicated that A2AR was associated with key biological mechanisms linked to tumor evasion and disease progression. *In vitro* blockade of A2AR led to a notable reactivation of viability and proliferative activity in CD8+ T cells. High-throughput virtual screening identified a small molecule, which was named AB-1, acting as a potential inhibitor of A2AR. Interestingly, AB-1 mediated enhanced IFN-γ expression i*n vitro* and significant anti-tumor activity *in vivo*. Given these findings, the A2AR blockade can restore T cell activity, enhance the effectiveness of checkpoint inhibitors, and reprogram the TME to support an effective immune response. In summary, A2AR represents a promising therapeutic target for overcoming immune evasion in the TME of cancers such as breast carcinoma and glioma.

505. 646. **NEUTROPHILS KEY ROLE IN ACUTE LOCAL RESPONSE INDUCED BY THE MELANOMA VACCINE TRIMELVAX: IMPLICA?ONS FOR TUMOR REJEC?ON**

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TRIMELVax is an innova=ve immunotherapeu=c vaccine for malignant melanoma, which combines heat shock-condi=oned human melanoma cell lysates with a mollusk hemocyanin as an adjuvant. Preclinical studies demonstrated significant e□ec=veness in reducing tumor growth and improving survival in mice with melanoma and colorectal tumors. However, the precise immunological mechanisms behind its an=-tumor e□ects have remained unclear. This study focused on understanding how TRIMELVax induces inflamma=on at the injec=on site, recruits innate immune cells, and triggers DC migra=on to draining lymph nodes. To achieve this, C57BL/6 mice were injected in the footpad with TRIMELVax, PBS, or control vaccines, and then the skin and the popliteal lymph node were harvested for qPCR or FACS analysis. Results showed that TRIMELVax rapidly induced a specific pa;ern of proinflammatory cytokines and chemokines, leading to an acute innate immune response in the administra=on site. Neutrophils, type 1 macrophages, monocytes, cDC1, Langerhans cells, and monocyte derived-DCs were recruited to the footpad, while type 2 macrophages decreased. This early inflamma=on facilitated a superior migra=on of cDC1 and a new subtype of an=gen-presen=ng neutrophils to the lymph node compared to controls. We demonstrate that vaccine-induced neutrophils play a fundamental role in TRIMELVax's mechanism of ac=on. Deple=ng neutrophils reduced DC migra=on, especially cDC1, to the draining lymph node and suppressed TRIMELVax's ability to control tumor growth. In summary, TRIMELVax triggers a rapid and potent ac=va=on of the innate immune system, which seems crucial

for a more e□ec=ve adap=ve immune response against aggressive melanoma tumors. These results underscore the promise of TRIMELVax as a poten=al immunotherapeu=c approach for melanoma treatment.

506. 655. **CHARACTERIZATION OF THE T CELL IMMUNE LANDSCAPE IN OVARIAN CANCER MODELS WITH DIFFERENT DNA REPAIR CAPACITY: IMPLICATIONS FOR POTENTIAL COMBINATION THERAPIES**

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Ovarian cancer (OC) is one of the most lethal tumors in women, characterized by aggressiveness, late diagnosis, and limited treatment effectiveness. DNA repair mechanisms, particularly homologous recombination (HR), are crucial in the response to DNA-damaging therapies like cisplatin and PARP inhibitors. Approximately half of high-grade ovarian cancer patients have HR deficiencies associated with mutations in BRCA1, BRCA2, and TP53. This study aimed to investigate how tumor HR capacity influences immune responses, with the goal of improving combination therapy strategies. For this, we characterized the T-cell immune landscape of two murine models of ascitic OC obtained through intraperitoneal inoculation of 5x10^6 HR-proficient (WT) and HR-deficient ($p53^-$/$BRCA2^-$) ID8 cells into C57BL/6 mice. When animals showed abdominal distension (10.5 ± 0.72 and 6.83 ± 0.70 weeks, respectively), the distribution of T-cell subsets in ascites and spleens was analyzed by flow cytometry. Results show that HR-deficient (HRd) and HR-proficient (HRp) tumors have a similar proportion of infiltrating immune cells (CD45+). Both models also exhibit a similar distribution of CD4+ T-cells. However, the HRd model showed a lower proportion of total cytotoxic T cells both in ascites ($p<0.05$) and spleens ($p<0.01$), but a higher percentage of activated CD8+ T-cells ($p<0.01$ spleen; $p<0.001$ ascites) and a lower proportion of exhausted Tim3+ T-cells ($p<0.001$ spleen; $p<0.05$ ascites) than HRp tumors. Additionally, we assessed the levels of cytokines involved in OC progression and immune cell infiltration in the ascitic fluid. Only IL6 ($p<0.05$), involved in tumor aggressiveness, and CXCL10 ($p<0.01$), secreted as a response to genomic instability, were detected at higher levels in HRd ascites compared to HRp. The increased activation and reduced exhaustion of CD8+ T-cells in HRd tumors suggest a favorable environment for combination therapies with immunotherapy, which are crucial for extending patient survival. This study offers valuable insights for optimizing immunotherapy strategies in OC.

507. 663. **AN *IN VITRO* MODEL FOR MODULATING T LYMPHOCYTES VIA PLATELETS**

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Background: Traditionally known for their essential role in blood clotting, platelets have recently gained attention for their involvement in inflammatory and immune responses. The platelet-to-lymphocyte ratio (PLR) is often elevated in cancer patients, suggesting that tumor cells can influence platelet activity, which may, in turn, affect the antitumor immune response. **Objectives:** this study aimed to develop an *in vitro* system to investigate the impact of platelets on the functional state of T lymphocytes. **Methods:** Platelets and peripheral blood mononuclear cells (PBMCs) were isolated from EDTA-anticoagulated blood of healthy donors using centrifugation and Ficoll-Paque gradient separation. Co-cultures of platelets and T lymphocytes were set up to explore various conditions, including PLRs of 50 and 100, as well as pre-treatment of platelets with 2mM $CaCl_2$ or co-culture with tumor cells at a ratio of 1:1000 (tumor cells:platelets, MEL-XY2 melanoma cell line). The effects of pre-treatment on platelets were assessed by flow cytometry, focusing on activation and differentiation markers CD62p and Annexin-V. Following an overnight culture, the expression of several T-cell activation and exhaustion markers were analyzed by flow cytometry as well. **Results:** Pre-treatment of platelets significantly increased Annexin-V expression. When PBMCs were co-cultured with pre-treated platelets—either with $CaCl_2$ or tumor cells—at a PLR of 50, there was a two-

fold increase in the expression of activation markers HLA-DR$^+$ and CD69$^+$ on CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ lymphocytes relative to the untreated control. **Conclusion:** This study introduces an *in vitro* model that facilitates the examination of how platelet modulation can affect T lymphocytes functionality, allowing future investigation on a potential role of platelets in the antitumor immune response.

508. 667. **DIFFERENTIALLY SECRETED FACTORS IN VEMURAFENIB-RESISTANT MELANOMA CELLS: AN EXPLORATORY STUDY ON POSSIBLE MOLECULES THAT MODULATE MACROPHAGES**

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The incidence of melanoma, the most aggressive skin cancer, has surged rapidly over the past decade. Although targeted therapies using BRAF inhibitors such as vemurafenib and dabrafenib have shown an impressive initial success in patients that display the BRAFV600E mutation, their effectiveness is often short-lived as tumors quickly develop resistance. Our previous research demonstrated that vemurafenib-resistant melanoma cells exhibit decreased autophagic flux, which is linked to an upregulation of the secretory pathway, thereby facilitating resistance transfer to sensitive cells. Here, we aimed to identify potential molecules secreted by melanoma cells that modulate macrophage activation. First, macrophage-derived THP-1 cells were cultured in conditioned media (CM) obtained from vemurafenib-sensitive (LuS) and resistant (LuR) Lu1205 melanoma cells. Only macrophages exposed to CM-LuR showed increased IL-10 and TIM-3 expression, though both CM induced higher levels of Galectin-9 (Gal-9). Then, we analyzed the secretome of LuS and LuR cells using mass spectrometry. LuR cells showed increased secretion of proteins associated with negative regulation of apoptosis, cell adhesion and cytoskeleton organization. Additionally, LuR cells exhibited elevated Gal-7 secretion, which is known to promote IL-10 release, a hallmark of M2 macrophages. Interestingly, only resistant cells secreted TGF-beta, an important modulator of TIM-3 expression and alternative macrophage activation. Next, we investigated the expression of TIM-3 ligands (Gal-9 and HMGB-1) in sensitive and resistant cells. Notably, whereas expression of HMGB1 was high and similar in both cell lines, Gal-9 was undetectable. Given that HMGB1 stimulates Gal-9 release in macrophages through TIM-3 and TLR4 signaling, this may account for the comparable levels of Gal-9 observed in macrophages. These findings suggest that factors secreted by vemurafenib-resistant cells may not only affect resistance of nearby melanoma cells but also alter macrophage phenotype, thereby reducing antitumor immunity.

509. 669. **VISMODEGIB LOADED LIPOSOMES: A TOPICAL ADMINISTRATION WAY OF REPURPOSING VISMODEGIB FOR THE TREATMENT OF SKIN CANCER**

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Background: Vismodegib, an inhibitor of the Hedgehog pathway (active in certain tumors), was first approved in 2012 for treating basal cell carcinoma. It is the preferred treatment for non-resectable or recurrent lesions, but it can lead to severe systemic side effects. Its efficacy in treating different skin malignancies, such as melanoma, squamous cell carcinoma, or cutaneous T-cell lymphoma, has not been evaluated. We propose repurposing Vismodegib to treat skin malignancies through topical application using nanotechnology. **Objectives**: We aim to evaluate the efficacy of Vismodegib-loaded nanosystems topical administration on melanoma growth, compare it with oral treatment, and analyze tumor inflammatory infiltration. **Methods**: *In vitro* proliferation inhibition: B16 cells were plated at low density (104 cells/p96 well), treated with Vismodegib (100-0.097μM) for 24 hours, and evaluated

for viability (Alamar blue staining).
Topical treatments: Vismodegib was encapsulated in ultradeformable liposomes (UDL) and conventional liposomes (CL). CL were combined with ethosomes (Etho) to enhance penetration. *In vivo* assay: C57BL/6 mice were intradermally challenged with 10 B16 cells and treated after tumors were detectable. Non-treated and oral Vismodegib controls were included. Tumor size was measured over two weeks. Tumors were isolated and stained with H&E to evaluate inflammatory infiltration. **Results**: Vismodegib inhibited melanoma growth *in vitro* (50 µM, p<0.0001 vs. control). In vivo, delayed growth was also observed in the UDL-vismodegib group (699.5 vs. 1021.9 mm3 in the control group), together with a better general status of the animals. The LC-vismodegib+Etho group showed no difference in tumor size vs. control. Inflammatory infiltrated area in UDL-treated animals was increased compared to non-treated and LC-treated animals (30.5% of tumor area infiltrated vs 21.4% and 20.2%). **Conclusion**: Vismodegib-UDL topical application represents a novel alternative for treating cutaneous malignancies. Vismodegib use in melanoma may represent a novel target for tumor growth inhibition and potentiation of the immune response.

510. 683. **4-METHYILUMBELLIFERONE GENERATES A POSITIVE MODULATION ON IMMUNE CELLS INVOLVE IN ANTI-GLIOBLASTOMA RESPONSE**

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Glioblastoma is the most aggressive primary brain tumor. The immune response to glioblastoma starts with microglial cells. Subsequently, the disruption of the blood-brain barrier allows neutrophils, γδ T and NK cells to infiltrate the tumor. Temozolomide (TMZ), the standard treatment, has limited efficacy and significant adverse effects, highlighting the need for new therapies. Previously, we have demonstrated the anti-tumor effect of 4- methylumbelliferone (4MU) on glioblastoma cells. To evaluate the effects of 4MU compared with TMZ on immune cell populations involved in the anti-GBM response, we used BV2 microglial cells, and neutrophils, γδ T, NK and myeloid-derived suppressor cells (MDSCs) from healthy donors. We assessed metabolic activity, cell proliferation and cell death through the XTT assay, BrdU and PI incorporation, respectively. Cytokines were measured using ELISA. NK cell cytotoxic activity was evaluated on PBMC co-cultured with K562 or GBM cells as targets, assessing cell death by flow cytometry (FC) and immunofluorescence. CD69 expression on γδ T cell was assessed by FC as an indicator of activation. The neutrophil respiratory burst was evaluated by FC, using dihydro-rhodamine 123. NETs release was evaluated by IF. The effects of drugs on MDSCs population were assessed by FC. Our results showed that TMZ induced the death of 40% of BV2 cells, while 4MU caused only 17% at higher doses (p<0.0001). Also, 4MU increased IFN-γ and IL-12 secretion by these cells, whereas TMZ only increased IL-12. On neutrophils, TMZ increased cell death while 4MU didn't, but induced an increment in NETs release and oxidative stress (p<0.05). Notably, γδ T cells remained unchanged by TMZ treatment, while 4MU activated them (p<0.05). Similarly, 4MU enhanced NK cell activity, while TMZ reduced it (p<0.05). Furthermore, 4MU decreased MDSCs population (p<0.05). In summary, 4MU shows potential as a beneficial immune modulator supporting the value of this drug in GBM therapy.

511. 687. **NATURAL KILLER ARTFICIAL CELL-DERIVED VESICLES: INNOVATIVE NK-EXTRUSOME AND ALTERNATIVE NANODRUG DELIVERY SYSTEM FOR LUNG CANCER**

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Background: Lung cancer (LC) has the highest mortality rate worldwide. Its pathogenesis is multifactorial, and while targeted therapies are recommended, many patients must rely on classical treatments (e.g., chemo-immunotherapy) which have limitations. Extrusomes (EXT), artificial cell-derived vesicles (ACDVs), have emerged as a promising tool for overcoming clinical translation barriers. This study proposes a formulation of a chemotherapeutic- encapsulated in EXT generated from human natural killer (NK) cells (DTX-EXTs) and evaluates their cytotoxic effects and internalization mechanism on LC cells. **Hypothesis:** DTX-EXTs derived from NK cells exhibit superior anti-tumor efficacy compared to conventional extracellular vesicles (EVs) and unmodified EXTs in LC cells **Methods:** EXT were generated by cell extrusion. Morphology was analyzed using TEM. Stability was determined via zeta potential. EXT and EVs composition was determine via proteomic analysis. The cytotoxic effect was assessed using MTT and caspase 3/7 activation assays via flow cytometry on human LC cell lines A549 and NCI-H1975. Cellular uptake mechanisms were explored with pharmacological inhibition of membrane fusion, macropinocytosis, and clathrin/caveolae-mediated endocytosis. **Results:** All vesicles displayed a cup-shaped morphology (mean size of <200 nm) and stable composition. Proteomic analysis revealed differentially present proteins associated with EVs and NK cell characteristics markers, also presented distinct protein enrichment pattern. A significant cytotoxic effect was observed with EXT-DTX treatments compared to EXTs and EVs in both cell line, with more efficacy in A549 cells, apoptosis induction corroborated these findings. Internalization studies identified perinuclear localization and involvement of clathrin- mediated as a primary mechanism in all group of vesicles. **Conclusion:** This study offers the first in-depth analysis of docetaxel-loaded extrusomes derived from NK cells, demonstrating their promise as a novel therapeutic delivery platform with enhanced anti-tumor activity. Additional studies are required to further assess the therapeutic potential and safety of DTX-EXTs for cancer treatment.

512. 689. MHC CLASS I POLYPEPTIDE-RELATED SEQUENCE A (MICA) VARIANTS: MECHANISMS OF CELLULAR RELEASE AND THEIR EFFECTS ON THE REGULATION OF THE NKG2D RECEPTOR ON NK CELLS

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MICA is a stress-induced membrane ligand engaged with NKG2D receptors on NK cells activating the cytotoxic response. However, this molecule can be released in a soluble form by metalloprotease action or bound to extracellular vesicles(EVs),which would have a down-regulator effect on NKG2D,altering the cytotoxicity of NK cells.There are multiple MICA protein variants, whose biochemical characteristics and immunological effects have been studied only in a limited number of proteins. Our main objective was to study the cellular mechanisms of release and effects of MICA variants on the NKG2D receptor on NK cells. Methodologically, biochemical and cellular approaches were used in both transfectant systems with MICA variants (*002,*008,*009,*011and*019)and tumor cell lines.The release mechanisms were evaluated using metalloprotease inhibitors. The effect of MICA variants on the NKG2D expression was analyzed by flow cytometry. We describe that

several alleles of MICA, other than the previously described MICA*008, can be recruited to EVs. Interestingly, MICA variants showed a differential release profile after the inhibition with metalloproteases. While MICA*008 was not affected, other variants were mainly recruited into EVs with a reduced release of soluble form. MICA*008 was reported to be recruited to EVs via its glycosylphosphatidylinositol (GPI) anchor, however, the other MICA alleles now found in EVs did not have a GPI, suggesting that a different cellular mechanism is required for their recruitment to EVs. Both soluble and EV forms of MICA could induce the downregulation of the NKG2D receptor. However, this effect would be more potent by the*002 and*008 variants bound to EVs. These results indicate,for the first time,the differential release mechanisms, and down-regulatory effects on NKG2D receptors by MICA variants.Also, these findings would be important when selecting patients to be treated with anti-MICA antibodies that avoid the release of MICA from the membrane,whose variants could be released through EVs,augmenting the immune evasion mechanisms.

513. 713. **CTLA4 GENETIC VARIANTS ASSOCIATED WITH UROTHELIAL BLADDER CANCER SUSCEPTIBILITY**

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Introduction: Urothelial bladder carcinoma (UBC) is the most prevalent histological type of bladder cancer. The CTLA-4 was the first immune checkpoint to be clinically targeted for cancer treatment. *CTLA4* variants have been related to higher soluble CTLA-4 expression and may be a biomarker of choice for immunotherapy treatment. *CTLA4* rs231775 (+49A>G) and rs231779 (+1822C>T) variants in UBC and their influence on tumor presence and progression is not fully understood and present conflicting results. **Objective:** To evaluate the association between the *CTLA4* rs231775 (+49A>G) and rs231779 (+1822C>T) variants and susceptibility, stage, prognosis, and response to treatment of the UBC. **Methods:** The study included 145 controls and 140 patients with UBC undergoing surgery. The patients were stratified as non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MICB), metastasis, recurrence, low/moderate/high/very high risk. The *CTLA4* variants were determined using real-time polymerase chain reaction and the genotypes were tested in the allelic, codominant, dominant, recessive, and overdominant genetic models. **Results:** The UBC patients were older and mostly smokers, with greater waist circumference, systolic, and diastolic arterial pressure ($p<0.001$, $p=0.005$, $p=0.006$, and $p<0.001$, respectively) than controls. Patients carrying the AG genotype (rs23177 A>G) and those with the CT genotype (rs231779 C>T) showed a lower chance of presenting UBC than those with other genotypes [odds ratio (OR=0.40; 95% confidence interval (IC): 0.16-0.98, $p=0.045$) and rs231779 (OR=0.35; 95% IC: 0.14-0.87, $p=0.024$). R^2 Nagelkerke analysis demonstrated that a model with age and smoking added to the *CTLA4* rs231775 variant explained 77.0% of the susceptibility to UBC and a model with age and smoking added to the *CLTA4* rs231779 explained 77.2% of the susceptibility to UBC. **Conclusion:** The heterozygous genotype of both *CTLA4* rs231775+49A>G and rs231779 +1822 C>T, in the overdominant model, together with age and smoking may be useful as potential biomarkers for the UBC susceptibility.

514. 731. **THE ROLE OF PANX1 IN MODULATING THE TUMOR MICROENVIRONMENT AND PROGRESSION OF COLON CANCER**

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Background: Colon cancer is one of the leading causes of cancer-related mortality worldwide. Increasing evidence suggests that the tumor microenvironment (TME) plays a critical role in cancer progression. PANX1 is a hemichannel that is overexpressed in colon cancer, known to release ATP, affecting the TME. Understanding the relationship between PANX1 expression and TME composition could provide insights into its role in colon cancer progression. **Aim**: To evaluate the relationship between PANX1 expression with colon cancer progression and TME composition. **Methods**: The relationship of *PANX1* mRNA expression with prognosis was evaluated using TCGA-COAD and GTEx databases. Correlation between *PANX1* expression and immune cell markers, as well as the ATP receptors, were evaluated using TIMER2.0. Immunohistochemistry and immunofluorescence were performed to evaluate the co-expression of PANX1 with CD8+ T cells and CAFs in tumor samples (n = 5). **Results**: PANX1 was overexpressed in tumor samples compared with adjacent tissues using public databases and local samples. Elevated *PANX1* mRNA expression in tumors correlated with worse disease-free survival. *PANX1* expression levels were significantly correlated with *CD8*+ T cells, CAF and neutrophil markers. Additionally, *PANX1* expression demonstrated a strong correlation only with the ATP receptor *P2RX7*. Immunofluorescence in tumor tissues confirmed the correlation of PANX1 with CD8+ T cells and CAFs, further supporting the hypothesis that PANX1 influences the composition of the TME. **Conclusions**: These findings highlight PANX1 as a potential target for therapeutic strategies aimed at altering the TME to improve colon cancer prognosis. **Acknowledgement**: Fondecyt 11190990, Internal funding from Universidad Finis Terrae FF2023.

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515. 164. **IMPACT OF ANGIOTENSIN II ON THE CELL COMMUNICATION AT THE MATERNAL-FETAL INTERFACE THROUGH EXTRACELLULAR VESICLES**

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Background: Extracellular vesicles (EV) are cell-derived particles that contain molecules serving as key mediators in cell-cell communication. Angiotensin II (Ang II) has proven to modulate the interaction between decidual stromal (DSC) and extravillious trophoblast cells (EVT). Whether EVs from DSC modulate trophoblasts functions under the influence of Ang II is still unknown. Objective: To evaluate the effects of EV isolated from Ang II-treated DSC on EVT functions and immune cell activation. Methods: Endometrial stromal cell line St-T1b was decidualized (DC) *in vitro* with dibutiryl-cAMP and medroxyprogesterone acetate and treated with Ang II for the last 48h. mRNA of DC marker (PRL, IGFBP-1, FOXO1, vimentin) expression was evaluated by RT-qPCR, and type-1 angiotensin receptor (AGTR1) expression by immunofluorescence. EVs were isolated from St-T1b supernatants by ultracentrifugation and characterized by western blot, nanoparticle tracking analysis and cryo-TEM. Swan-71 and HTR-8/SVneo cells were stimulated with EVs, and their migration and invasion were evaluated. EV uptake by peripheral blood mononuclear cells (PBMCs) and NK cell activation was assessed by flow cytometry. Results: Ang II-DC-St-T1b cells express high levels of AGT1R in comparison with DC-St-T1b. Large (lEV) and small (sEV) fractions were isolated. EVs from Ang II-treated cells increased Swan-71 and HTR-8/SVneo migration and invasion. lEV increased Swan-71 but decreased HTR-8/SVneo invasion. sEV decreased Swan-71 but increased HTR-8/SVneo invasion. EV were uptaken mostly by $CD14^{+}HLADR^{+}CD16^{-}$ cells and by $CD3^{+}$ and $CD3^{-}CD16^{+}$ cells. No significant effect was observed in NK markers CD158 and CD94 expression. Conclusion: Ang II enhances the expression of AT1R and decidualization markers in endometrial cells. This may influence

the release of EVs which can transport RAS elements that can be taken up by trophoblasts, affecting their migratory/invasive capacity during embryo implantation and placentation. Furthermore, EVs could modulate immune responses by maternal monocytes and lymphocytes, which is still unclear and needs further clarification.

516. 208. CHRONIC EXPOSURE TO GLYPHOSATE-BASED HERBICIDES COMPROMISES SEMEN QUALITY, WHICH IN TURN DISRUPTS THE IMMUNOREGULATION OF THE FEMALE GENITAL TRACT AND AFFECTS FETAL DEVELOPMENT

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Background: The widespread use of glyphosate-based herbicides (GBH) has generated significant concerns about their potential impact on reproductive health. Despite these concerns, much of the existing data remains controversial and is largely derived from in vitro and animal studies, leaving a gap in our understanding of GBH's effects on male fertility in humans. **Objectives:** To study sperm quality and semen inflammation in men chronically exposed to GBH and controls. Additionally, an experimental animal model was used to investigate the effects of GBH on sperm quality, male fertility potential, and embryonic and placental development.**Methods:** Sperm quality in human patients was assessed following the WHO guidelines. Semen levels of cytokines/chemokines and leukocytes were assessed by flow cytometry. In parallel, C57BL/6 male mice were orally treated with 400 mg/kg/day of GBH or water (controls) for 35 days. After that, males were mated with BALB/c females. Sperm quality was analyzed after euthanizing males on day 42. Uterine immune cell infiltration was assessed at the peri-implantation window and different fertility parameters were evaluated on gestational day 19. Statistical analysis was performed using the Mann-Whitney test.**Results:** Significant reduced sperm concentration, motility, and normal morphology as well as semen inflammation, characterized by increased leukocyte counts and altered seminal plasma composition, were observed in GBH-exposed men. Similarly, GBH-exposed mice exhibited decreased semen quality. Interestingly, females mated with these males showed marked uterine inflammation during the peri-implantation period, evidenced by increased infiltration of different leukocyte subsets. Strikingly, pups sired by these males showed altered embryonic and placental development, with lower fetal and placental size/weights. **Conclusion:** These findings suggest that chronic exposure to GBH not only impairs semen quality in both humans and rodents but also alters the physiologic ability of semen to induce immunoregulation and compromises fetal development.

517. 225. ANALYSIS OF IMMUNOENDOCRINE PARAMETERS IN SEMINAL PLASMA AND FERTILITY MARKERS IN PATIENTS UNDERGOING ASSISTED REPRODUCTIVE TREATMENTS

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Infertility affects 15% of couples, with the male factor contributing to approximately half ofthe cases. The relationship between immunoendocrine parameters in seminal plasma and fertility markers in patients undergoing assisted reproductive treatments (ART) is an area of growing interest, given the impact that the balance between the immune and endocrine systems can have on the success of these procedures. In this study, the levels of testosterone, cortisol, and the cytokines IL-1b and IL-8 in seminal plasma were analyzed in relation to ART outcomes and their possible association with fertility markers, including 5'tRF-Gly. This small RNA, which regu-

lates various physiological and pathological processes, has shown significantly elevated levels in previous studies conducted in our laboratory among individuals who did not achieve pregnancy. A prospective study was conducted on couples undergoing ART with donated oocytes at the PROAR Medical Center between 2018 and 2022. Seminal plasma samples were collected from normozoospermic men, classified according to ART outcomes (ICSI (+) n:39,ICSI(-
) n:25). Cytokine and hormone levels were quantified using ELISA, and 5'tRF-Gly levels were measured by SLO-RTqPCR. The results did not show significant differences in immunoendocrine parameters with respect to ART outcomes. However, a significant correlation was observed between IL-1b and cortisol (p=0.002, Spearman r) and between IL-8 and testosterone (p=0.03, Spearman r) in patients with a normal body mass index (BMI). Those patients who achieved pregnancy had an inverse correlation between IL-1b and 5'tRF-Gly levels (p=0.002, Spearmanr). In patients with multiple ART failures, a correlation was identified between IL-1b levelsandthe fertilization rate (p=0.04, Spearman r), as well as an inverse correlation between IL-8 andsperm vitality percentage. In summary, these preliminary findings provide valuable insights into the role of immunoendocrine parameters in male fertility.

518. 253. **EMBRYO QUALITY CONDITIONS TOLEROGENIC RESPONSE AND ENDOMETRIAL RECEPTIVITY DURING THE PERI- IMPLANTATION PERIOD**

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To achieve successful embryo implantation, adequate decidualization of endometrium and a competent embryo are required. Although endometrial stromal cells play an essential role as "multitasking superstars" since conditioning immune cells and interacting with embryo. However, the impact of embryo secretome on implantation is poorly known. Our aim is to study the endometrial-embryo-immune interactome, focusing on embryo quality biosensing. First, we performed bioinformatic analysis using data obtained from public databases, focusing on protein-protein interactions between a competent blastocyst's secretome and a receptive endometrium. A network with specific genes involved in embryo attachment and cell-cell junctions was obtained. Therefore, using atomic force microscopy and spectroscopy (AFM-FS), we investigated the impact of human embryo-conditioned media (ECM) obtained from normal (ND) or impaired developing (ID) embryos on the nanomechanical properties of decidualized stromal cell line (HESC). Stromal cells treated with decidualization stimuli (MPA+db-cAMP for 8 days) showed a significant decrease in cellular stiffness, suggesting that they would be more permissive to embryo implantation. Interestingly, decidualized stromal cells exposed to ECM from ID embryos were stiffer. Considering that regulatory immune cells actively participate in embryo implantation, and that the bioinformatic analysis revealed interactions involving cytokine-signaling, we proposed to evaluate whether embryo secretome could alter the profile and function of mononuclear cells, particularly dendritic cells (DCs). Peripheral monocytes isolated from healthy women were differentiated to immature DCs (rhGM-CSF+rhIL-4) in the absence or presence of ECM for 5 days. ECM from ND embryo induced an HLA-G$^+$ tolerogenic profile on DCs ($p<0.05$, Wilcoxon Test). Moreover, DCs treated with ID-ECM not only showed a CD86$^+$ pro-inflammatory profile but also significantly impaired trophoblast migration in an embryo implantation in vitro model. These results suggest that soluble factors secreted by embryo might have an impact on endometrial receptivity, affecting mechanical properties of stromal cells and the functionality of resident immune cells.

519. 275. **ALTERATIONS IN ENDOMETRIAL PROGRAMMING LED TO RECURRENT IN VITRO FERTILIZATION FAILURES: FOCUS ON INFLAMMATORY RESPONSE AND ANGIOGÉNESIS**

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The decidualization program entails changes in the secretome of endometrial stromal cells allowing a sterile inflammation associated with successful embryo implantation. The mechanisms that lead to alterations in this process associated with recurrent implantation failures (RIF) are still unknown. Our goal was to evaluate alterations in the decidualization program that influence decidual cells' functions and inflammatory response, affecting implantation. First, we performed a bioinformatic analysis based on standardized pathways focusing on processes related to implantation and immunoregulation; then, we validated the expression of genes involved in these processes in endometrial biopsies. We detected decreased expression of decidualization markers (*IGFBP1* and progesterone receptor), altered expression of molecules associated with blastocyst adhesion (*MUC1* and *ITGA8*) and an imbalance between pro/anti-angiogenic factors in RIF samples compared to fertile women ($p<0.0001$, Mann-Whitney test). Interestingly, we found a reduction in *NLRP3* expression ($p<0.0001$) and a decreased frequency of IL-1β-producer stromal cells was observed by flow cytometry ($p<0.05$). Focusing on mechanisms related to these alterations and considering that IL-1β production is associated with the activation of endoplasmic reticulum (ER) stress sensors and unfolded protein response (UPR), we tested the expression of ER stress sensors. We confirmed a decrease in ER stress/UPR mediators in RIF biopsies. Since both processes are related to embryo implantation, we tested the effect of RIF stromal cells on trophoblast migration. We observed a decreased trophoblast migration index accompanied by decreased ER stress response in RIF primary cultures. In fact, when we induced pharmacological ER stress on RIF stromal cells, they were able to respond changing their secretome, therefore, modifying stromal cells functions in pathways involved in vascular tubulogenesis and angiogenesis. Taken together, these results suggest that RIF patients display alterations in the decidualization program that might have negative impact on the induction of physiological inflammation associated with alterations in ER stress response.

520. 287. **CHARACTERIZATION OF THE ADAPTATIVE IMMUNE RESPONSE IN HIV EXPOSED UNINFECTED INFANTS**

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Despite the worldwide success avoiding vertical HIV transmission, *in utero* viral exposure causes significant alterations neonates development. Consequently, these HIV exposed uninfected (HEU) newborns have higher rates of opportunistic infections, morbidity, and mortality. Additionally, limited response to vaccination has been reported, as T helper (Th) cells proliferate and produce cytokines less after restimulation. Interestingly, HEU show inflammatory basal cytokine profile, reduced thymic size with less production of total T cells, and reduced specific antibody concentrations. These observations suggest that HEU infants born with an altered immune system, which would explain its increased infections susceptibility. Thereby, we evaluated, in HEU newborns, the total immunoglobulins concentrations, the frequency and phenotype of B, Th and dendritic cells, and functionality markers, as CD71, HLA- DR, CD69, CD80 and CD86 as well as cytokine production. Also, Th cells, were stimulated, and differentiated to Th1 profile and reevaluated. Our results showed important alterations in IgG antibodies concentration in HEU newborns which are sustained along the first life year, showing higher IgG1 and IgG3 concentrations and lower IgG2, like their HIV+ mothers´. Regarding B cells, minor differences in the proportions of B lymphocyte subpopulations, but less phenotypic differentiation and lower proliferative capacity was found. On the Th cells compartment, we observed HEU infants born with less proportions of Th1, Th2, Th17 and Th1/17 cells, also they had less ca-

pacity to secrete IL-2 and IL-4 under polyclonal stimulation. Interestingly, under Th1 differentiation conditions they produced higher amounts of INF-γ. Even though dendritic cells, myeloid and plasmacytoid, were found in similar proportions to control groups, they possess more activated phenotypes, expressing higher levels of CD80 and CD86; this phenotype correlated inversely with all the Th cells proportions. In conclusion, our results suggested that *in utero* exposure to HIV generates a fetal programming that affects the development of its immune defense.

521. 302. **MATERNAL-PATERNAL IMMUNE CELLS CO-CULTURE DIFFER IN THEIR NR4A EXPRESSION PATTERN COMPARING URSA PATIENTS AND CONTROLS**

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Spontaneous abortion is the most common complication of early pregnancy. Three or more consecutive losses define a recurrent situation. A majority of these losses remain unexplained (URSA). As immunological mechanisms seem to participate in their development, medical centers worldwide set up and apply intra-dermal injections of paternal mononuclear cells through diverse protocols referred as “Paternal Immunotherapy”. The treatment effectiveness is followed by measuring the initial and post-treatment blocking activity in a mixed lymphocyte reaction: maternal and paternal mononuclear cells are co-cultured in the presence of maternal serum. NR4A orphan receptors subfamily is integrated by three immediate early genes functioning as ligand independent transcription factors. We have previously reported that patients show significant lower mRNA expression levels of NR4A2 and NR4A3 nuclear orphan receptors, when compared to control fertile women. To further advance in the study of the NR4A potential involvement within the numerous mechanisms responsible for the maternal tolerance towards the embryo, we set up a maternal and paternal mononuclear cells co-culture to evaluate NR4A mRNA expression in this context. Mononuclear immune cells were maintained alone or combined 1:1 into standard culture medium, at 37°C, 5h, previous to determine NR4A mRNA expression by real- time PCR. Assuming the hypothesis that expression in co-culture might be close to the mean expression in individual cultures if no effect between heterologous cells, and that the diversity of our unexplained samples might include other unknown pathologies than immunological, we choose to compare average values for each gene/group. The URSA group showed co-cultures with lower mRNA expression for NR4A1 and NR4A2, and higher expression for NR4A3, than their respective individual cultures. Interestingly, Control group co-cultures showed opposite effects for NR4A1 (higher) and NR4A3 (lower) mRNA expression. These results strengthen our hypothesis of NR4A subfamily members participating as immune restrictive/promotive factors for embryo implantation and development.

522. 307. **RELATIONSHIP BETWEEN MATE CHOICE AND THE EXPRESSION OF BOVINE MAJOR HISTOCOMPATIBILITY COMPLEX GENES**

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3. Professor Edson Antônio Velano University

Fixed-time artificial insemination (FTAI) is a technique that has been employed in the reproduction of domestic animals. Despite facilitating reproductive management and reducing costs, this tool does not allow animals to choose their sexual partner. The ability to choose a sexual partner may be related to reproductive success. The major histocompatibility complex (MHC) gene complex is associated with immunological processes and, in recent decades, has been linked to reproductive success in mammals. The aim of this study was to evaluate the expression levels of MHC genes (*MHC*, *NC-1*, *NC-2*, *NC-3*, and *NC-4*) in bovine couples with successful reproduction, mated under two systems: inseminated animals (FTAI) and natural mating (NM). A total of 16 couples with successful pregnancies (10 NM and 6 FTAI) from the Hereford and Braford breeds were used. For gene expression analysis, firstly RNA was extracted from blood tissue, followed by cDNA synthesis, finally, it was performed the RT-PCR. The analysis was conducted in R Studio software, where outliers were checked, and normality and homoscedasticity of variances were evaluated. Upon detecting a violation of these assumptions, a Box-cox transformation was performed, and a mean comparison test at a 5% sig-

nificance level was carried out to identify which genes differed between the NM and FTAI groups. Gene expression in the FTAI couples was generally higher: *MHC*= 3.62[a], *NC-1*= 0.34[a], *NC-2*= 2.76[a], *NC-3*= 1.29[a], and *NC-4*= 1.67[a], than in NM: *MHC*=0.84[b], *NC-1*= 0.92[b], *NC-2*= 0.90[b], *NC-3*=1.12[a], and *NC-4*=0.97[a]. Significant differences (P<0.05) were observed in the expression of the *MHC*, *NC-1*, and *NC-2* genes between the NM and FTAI groups. These results indicate a possible relationship between MHC genes and reproductive success, which may be involved in mate choice and reproductive success in cattle.

523. 310. **EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX GENES ASSOCIATED WITH REPRODUCTIVE SUCCESS IN BEEF CATTLE**

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The major histocompatibility complex (MHC) consists of a set of genes primarily related to immunological processes, but in recent decades, it has been associated with reproductive success in mammals. The aim of this study was to evaluate the expression of MHC genes (*MHC*, *NC-1*, *NC-2*, *NC-3*, and *NC-4*) in bovine couples subjected to artificial insemination (FTAI) in two groups: a group of couples with successful pregnancy (GS) and a group of couples with failed pregnancy (GF). A total of 12 pairs (6 GS and 6 GF) of the Hereford breed were used. For gene expression analysis, firstly RNA was extracted from blood tissue, followed by cDNA synthesis, later, it was performed the RT- PCR. R Studio software was used for statistical analyses, and a Student's T-test with Bonferroni correction was conducted to compare means at a 5% significance level to identify which genes differed in their expression between the MN and FTAI groups. The results showed a significant difference at the 5% level (P<0.05) for the MHC (GS= 3.35 *vs* GF= 2.91) and *NC-4* (GS= 1.67 *vs* GF= 0.26) genes. It was observed that the GS group had higher expression levels of *MHC* and *NC-4* compared to the GF group. These results suggest that the expression of *MHC* and *NC-4* genes is associated with pregnancy success, as higher expression was observed in pairs that successfully produced a calf. These findings indicate that the *MHC* and *NC-4* genes may play a role in reproductive success in cattle, possibly acting at some stage of the conception and fetal recognition process.

524. 453. **SEMEN INFLAMMATION TO FERTILITY CHALLENGES: UNRAVELING THE IMMUNE CROSS-TALK BETWEEN MALE AND FEMALE GENITAL TRACTS AND ITS IMPACT ON OFFSPRING**

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Background: Male infertility accounts for up to 50% of all infertility cases, with urogenital inflammation, particularly chronic prostatitis, contributing to 15% of these cases. Chronic prostatitis patients show semen inflammation and reduced sperm quality. Given that the seminal fluid is crucial not only for fertilization but also for inducing immunoregulation un the female genital tract (FGT) to support embryo implantation and pregnancy development, chronic prostatitis could have further effects beyond semen inflammation. **Objective:** To investigate the impact of Experimental Autoimmune Prostatitis (EAP) on male fertility, immunomodulation in the FGT, and offspring development. **Methods:** C57BL/6 male mice were immunized with prostate antigens (PA) or saline (C) to induce EAP. The PA-specific immune response, prostate histopathology, and sperm quality were evaluated. Mating experiments with female BALB/c mice were performed to assess fertility parameters, uterine immune cell infiltration and cytokine/chemokine expression, and offspring development. Statistical analysis was performed using the Mann-Whitney test or ANOVA (p<0.05). **Results:** EAP males exhibited chronic prostate inflammation, semen inflammation and oxidative stress, and reduced sperm quality. Females mated with EAP males showed significantly increased uterine leukocyte infiltration, upregulated expression of IL-1β and IL-17A, along with reduced IL-10, and downregulated expression of embryotropic factors crucial for implantation and decidualization (LIF, IL-6 and IGFBP-1, respectively) whereas increased

expression of the blastomere pro-apoptotic factor TRAIL. Additionally, females mated with EAP males showed significantly lower fertility indexes, and higher rates of pre- and post-implantation embryo loss. Strikingly, offspring sired by EAP males exhibited reduced growth and decreased sperm quality when adults. **Conclusion:** Chronic prostatitis causes semen inflammation and disrupts immune regulation in the FGT, compromising fertility and negatively impacting offspring development. These findings highlight the need to properly diagnose and treat chronic prostatitis to safeguard fertility and prevent long lasting complications on the offspring.

525. 472. **BRUCELLA INFECTION ALTERS THE COMPOSITION AND FUNCTIONALITY OF SMALL EXTRACELLULAR VESICLES RELEASED BY THE PLACENTA**

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Small placental extracellular vesicles (sPEV) play a critical role in immunomodulation during pregnancy. *Brucella*, a zoonotic bacterium, is known to cause gestational complications in both animals and humans. This study assessed the impact of *Brucella abortus, B. melitensis*, and *B. suis* infections on the production, composition, and function of sPEV using ex vivo-infected human term placental explants. Eighteen hours post-infection, culture supernatants were collected, and sPEV were isolated by ultracentrifugation. The sPEV were characterized by particle count and hydrodynamic diameter, and their profiles were analyzed through nanoparticle tracking analysis (NTA). The effects of sPEV on the immune response of peripheral blood mononuclear cells (PBMC) were evaluated by stimulating these cells with different concentrations of sPEV for 24 hours, both in the presence and absence of *E. coli* lipopolysaccharide (LPS). Interleukin-6 (IL-6), IL-10, and TNF-α production were quantified by ELISA. NTA revealed a significant increase in the number of sPEV from infected explants compared to non-infected controls ($p<0.05$). Additionally, sPEV from infected explants were significantly smaller ($p<0.0001$). Both infected and control sPEV expressed CD63 and CD81 markers, either individually or concurrently, though CD63-positive sPEV were fewer in infected placentas ($p<0.05$). Non-infected sPEV induced a dose-dependent increase in IL-6, IL-10, and TNF-α production in stimulated PBMC, compared to unstimulated cells ($p<0.0001$). However, sPEV from *B. abortus*-infected placentas induced significant but lower increases in IL-6 and IL-10, with no rise in TNF-α levels ($p<0.05$). In contrast, sPEV from *B. melitensis* and *B. suis* induced only a slight increase in IL-6 at the highest dose tested ($p<0.05$). Placental infection did not impair the ability of sPEV to downregulate LPS-induced TNF-α and IL-10 production ($p>0.05$). Our findings demonstrate that *Brucella* infection alters the size, composition, and immunostimulatory properties of placental sPEV, potentially affecting placental-immune cell communication.

526. 565. **POTENTIAL PROTECTIVE EFFECT OF THE PROBIOTIC LENTILACTOBACILLUS KEFIRI CIDCA8348 IN A MURINE MODEL OF ENDOMETRITIS**

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Endometritis (E) is an inflammation of the uterine lining (endometrium), characterized by tissue damage, edema, leukocyte infiltration, and increased inflammatory mediators in the uterine cavity, which strongly affects reproductive capacity. *Lentilactobacillus kefiri* is a potentially probiotic microorganism obtained from the fermentation of kefir grains. We previously demonstrated that prophylactic treatment with *Lentilactobacillus kefiri* CIDCA8348 (Lk48) significantly reduces the rate of preterm birth, decreasing leukocyte infiltration and uterine tissue damage in a murine model of

the disease. The objective of our study is to evaluate Lk48's ability to attenuate E and improve reproductive capacity in mice. Virgin C57BL/6 female mice, aged 8-12 weeks, were pretreated intravaginally with Lk48 (10^8 UFC/ml) or milk (control) every 72 hours for 15 days. 24-72 hours after the last treatment, all females were challenged intravaginally with LPS (50 µg per mouse) to induce E and divided into two groups. In one group, uteri were collected 24 hours post-E induction and stained with hematoxylin and eosin (HE) for histological analysis. In another group, females were mated with BALB/c males 24 hours after E induction and sacrificed on day 12 of gestation to evaluate reproductive outcomes. Histological evaluations of control mice uteri revealed inflammatory exudate, abscesses, and a highly cellular lamina propria with the presence of neutrophils and plasma cells. In contrast, Lk48-treated mice exhibited no visible edema, leukocyte infiltrate, or lesions in the uterine epithelium. These uterine changes appear to impact reproductive capacity. While macroscopic analysis showed no embryonic resorptions on Lk48-treated mice uteri, 60% of the control group uteri exhibited at least one embryonic resorption, on day 12 of gestation (Fisher's exact test, p=0.1667).
In summary, *Lentilactobacillus kefiri* CIDCA8348 (Lk48) shows promise in alleviating LPS-induced endometritis in a mouse model and may improve reproductive outcomes. However, further research is needed to confirm its effectiveness.

527. 633. ALTERED B-CELL IMMUNOPHENOTYPE IN PREECLAMPSIA AND INTRAUTERINE GROWTH RESTRICTION: AN IMMUNOLOGICAL PROCESS

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Background: Preeclampsia (PE) and intrauterine growth restriction (IUGR) are clinically important obstetric complications. B cells play additional roles that contribute to both pregnancy-related well-being and pathologies. The pathophysiology of these diseases is complex and may be linked to the deregulation of B cell subpopulations. **Objective:** To evaluate the B cell immunophenotype in patients with preeclampsia and intrauterine growth restriction.
Methods: 15 pregnant women were included in three groups (controls (CO), PE and IUGR) with 5 patients each. The patients signed an informed consent letter. A 5 mL blood sample was taken by venipuncture. A density gradient was then performed to obtain PBMCs. The frequencies (%) of peripheral B cell subpopulations (CD19, CD38, CD27, CD24, IgD) as well as the activation markers CD69 and CD40 were determined by flow cytometry. **Results:** Total B cells did not differ between the three groups (p>0.05). However, it was observed that patients with PE tended to decrease their total B cells *vs.* CO (p=0.09). Peripheral B cell subpopulations (memory, plasmablasts, virgin, mature, double negative, pre-plasmablasts, transitional) also did not differ between the three groups (p>0.05). Transitional B cell and plasmablast subpopulations also showed a tendency to be decreased in pregnant women with PE *vs.* CO group (p=0.42 and p=0.22; respectively). Regarding activation markers, it is striking that the CD40 marker was decreased in B cells, where pregnant women with PE showed lower levels *vs.* CO (p=0.34). **Conclusions:** In PE, there is a tendency for peripheral B cells to decrease, where transitional B cell and plasmablast subpopulations could play an important role in the pathophysiology of PE, where the decrease in CD40 has to be studied in more depth.

SYSTEMS IMMUNOLOGY

528. 031. INTEGRATED SYSTEMS IMMUNOLOGY APPROACH IDENTIFIES PERSISTENT BLOOD MONOCYTE DYSFUNCTION INDUCED BY BOTH SYMPTOMATIC AND CLINICALLY SILENT PLASMODIUM VIVAX MALARIA

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1. Integrated systems immunology approach identifies persistent blood monocyte dysfunction induced by both symptomatic and clinically silent Plasmodium vivax malaria
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Background: Clinical immunity to *Plasmodium* parasites is acquired after extended exposure to the parasite. This form of immunity is not sterilising, with adults often experiencing asymptomatic infection. **Objectives:** Compared with *P. falciparum*, much less is known about the mechanisms modulating immunity to *P. vivax*. To address this, we pursued a systems immunology approach. **Methods**: We integrated high-dimensional mass cytometry, transcriptional profiling and clinical parameters of individuals experiencing symptomatic and asymptomatic *P. vivax* infection in an endemic area of Indonesia. **Results:** Symptomatic *P. vivax* infection featured transcriptional profiles of cell proliferation and fatty acid metabolism to support the expansion of T_{H1}-polarised T follicular helper cells and IgM^+ memory B cells (MBCs) involved in the control of parasitemia during acute infection. Unlike symptomatic *P. falciparum* malaria that induced a highly inflammatory response, clinical *P. vivax* infection featured the upregulation of anti-inflammatory pathways and check-point receptors, providing a feedback loop to ameliorate symptomatic infection. Furthermore, gene set enrichment analysis revealed a profound dysfunction of the blood monocyte compartment in symptomatic *P. vivax* infection, with inhibition of upstream regulators colony stimulating factor-1 and 2 predicted to be responsible for these processes. Transcriptional profiles supporting T cell differentiation, class-switched MBCs and a T_{H2} cell bias predicted reduced risk of clinical malaria in asymptomatic individuals. Despite these protective responses, monocyte dysfunction persisted in chronic asymptomatic infections of low parasitemia. **Conclusion:** The results identified monocyte dysfunction as a critical feature of *P. vivax* malaria. Our data suggest that asymptomatic *P. vivax* malaria is not innocuous as previously thought and might not support all immune processes to fully control parasitemia or efficiently respond to malaria vaccines.

529. 474. **DECODING THE IMMUNE LANDSCAPE OF CLEAR CELL RENAL CELL CARCINOMA: AN INTEGRATIVE BIOINFORMATIC APPROACH TO IDENTIFYING NOVEL THERAPEUTIC TARGETS**

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Background: Clear cell renal cell carcinoma (ccRCC) is one of the most common and aggressive forms of kidney cancer. The tumor microenvironment (TME) is critical in ccRCC progression and immune evasion. Despite advances in treatment, the molecular mechanisms underlying ccRCC remain poorly understood. **Objectives**: The aim of this study is to identify hub genes, differentially expressed genes (DEGs), immune cell participation and key pathways through bioinformatics tools to analyze gene expression data from ccRCC samples. **Methods**: Publicly available gene expression datasets of ccRCC were obtained from Gene Expression Omnibus. The analysis included data normalization, identification of DEGs through limma package in GEO2R, pathway enrichment analysis, gene set enrichment analysis (GSEA), gene set variation analysis (GSVA) and immune cell fraction quantification through CIBERSORTx. **Results**: A total of 39 samples were analyzed, the analysis identified 339 DEGs, pathway enrichment analysis revealed significant alterations in MAPK cascade pathway, TYROBP pathway and leucocyte activation pathway. MHC class II genes were downregulated in samples from patients with metastasis, specifically HLA-DQB1 and HLA DPA1. Network analysis showed CD48, CD74, CD247, HAVCR2, ITGAX, CXCR4 among others. Infiltrated immune cells fractions found T cells CD8, T cells follicular helper, T cells gamma delta and M2 Macrophages infiltrated in tumor samples, imputed from expression data. GSVA analysis found enriched pathways in each sample and clustered them based on the enrichment of said pathways, such as PD-L1 expression and PD-1 checkpoint pathway in cancer, which was found enriched in a set of samples. **Conclusion**: This integrative bioinformatics approach provides new insights into the molecular mechanisms of ccRCC, identifying potential biomarkers and therapeutic targets that could guide future research and treatment strategies.

530. 573. **EXPLORING THE IMMUNE-RELATED TRANSCRIPTOMIC PROFILES IN ISCHEMIC STROKE THROUGH INTEGRATIVE BIOINFORMATICS.**

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Background: Stroke remains a leading cause of death and disability, with the immune system playing a pivotal role in both the acute phase of injury and recovery process. Understanding the immune-related transcriptomic changes during these phases is key insight into potential biomarkers with clinical importance. **Objectives:** This study aims to uncover the immune related molecular signatures and pathways involved in stroke by analyzing transcriptomic data from blood samples during the acute phase. **Methods:** Transcriptomic profiles were extracted from the NCBI GEO repository, Differentially Expressed Genes (DEGs) were extracted using the limma package from GEO2R. Network analysis was conducted to reveal interactions between these DEGs and other significant pathways. Gene Set Variation Analysis (GSVA) was performed to explore enriched pathways related to immune responses in each sample. CIBERSORT was employed to quantify immune cell fractions, providing insights into the shifting of immune cell populations during stroke. **Results:** The systematic search of NCBI GEO repository found 6 databases with blood samples from patient and healthy controls with ischemic stroke, totaling 280 samples. The network built from the DEGs shared across databases and relevant DEGs from single databases revealed the altered pathways to be immune pathways, angiogenesis, IL-18 signaling, among others. Hub genes identified from network analysis revealed TIMP2, MAPK1, CD163, ARG1 and others. While GSVA explored enriched pathways in each sample, a pattern is yet to emerge as a characteristic signature in blood samples. Differences between immune cell fractions between healthy and stroke samples were found in some databases, but this pattern is not maintained across all of them. **Conclusion:** This integrative bioinformatics analysis shows the immune system's role in stroke and how changes across different phases of the disease can provide insights into the molecular changes in the disease.

TRANSLATIONAL IMMUNOLOGY

531. 033. ENHANCING TUMOR APOPTOSIS WITH THE COMBINED USE OF PENTOXIFYLLINE AND CHEMOTHERAPEUTIC AGENTS IN HODGKIN LYMPHOMA

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Background: Hodgkin lymphoma (HL) is a common B-cell neoplasm in adolescents and young adults (AYA). It is predominantly treated with chemotherapy, notably doxorubicin (DOX) and bleomycin (BLM), which are associated with severe adverse effects. Moreover, the cure rate decreases to 75% in advanced-stage patients. Thus, novel strategies are required to optimize treatment efficacy, minimize adverse effects, and improve clinical outcomes. Pentoxifylline (PTX) inhibits the NF-κB pathway and enhances chemotherapeutic-induced apoptosis in various cancer cell lines by increasing proapoptotic gene expression. Therefore, we propose PTX as a potential adjuvant therapy in HL treatment. **Objectives:** To evaluate the effect of PTX alone and in combination with chemotherapeutic agents on apoptosis in the Hs-445 cell line and in patients with HL. **Methods:** AYA patients with previously untreated HL were enrolled. They were randomized and blinded to receive either placebo or PTX (20 mg/kg/day) for two chemotherapy cycles. Clinical response was assessed by CT or PET-Scan, and

adverse effects were classified using Common Terminology Criteria and causality algorithms. Hs-445 cells were treated with PTX, DOX and/or BLM. Apoptosis, proliferation, and senescence were assessed through flow cytometry. **Results:** Half of the recruited patients achieved a complete response at the end of treatment, while the other half had a partial response. The most frequent adverse effects were nausea and vomiting, mostly grade II, possibly related to PTX. In vitro, PTX induced significant apoptosis compared to DOX and BLM, especially when combined with BLM and in triple therapy. PTX also had a potent antiproliferative effect, comparable to that induced by BLM and greater than DOX. Notably, PTX did not induce senescence and reversed senescence induced by DOX, and BLM. **Conclusion:** PTX is well tolerated in patients and is a potent inducer of apoptosis in HL, synergizing with DOX and BLM while reducing tumor proliferation and reversing senescence.

532. 159. **EARLY PREDICTION OF MORTALITY IN COVID-19 PATIENTS USING INTESTINAL PERMEABILITY MARKERS AND CYTOKINES**

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Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can present mild, moderate or severe symptoms, or critical symptoms that resemble those of sepsis. In the latter case, a deregulated immune response to SARS-CoV-2 infection leads to organ failure and to a high mortality risk. It has been suggested that sepsis involves an increase in intestinal permeability, which leads to greater microbial translocation from the intestine to the bloodstream, and hence to a higher inflammatory response in the patient. In this study, we measured the serum concentration of cytokines associated with the innate and the adaptive immune responses by bead-based immunoassays, and the serum concentration of molecules associated with the integrity of the intestinal barrier by ELISA, in patients with moderate, severe and critical COVID- 19. ROC curve results indicate that IL-6, IL-10, granulysin and sFas may be early biomarkers of fatal disease. In addition, we found that D-lactate, a metabolite produced by bacterial fermentation, and zonulin, a molecule that disassembles the tight junctions of the intestinal epithelial cells, were elevated in the serum of patients with severe COVID-19 and in those patients with secondary infections. The translocation of microbial components into the circulation appears to be related to the severity of COVID-19, as well as patient outcomes, as demonstrated by the principal component analysis. These results suggest that, in addition to cytokines, markers of intestinal permeability may be useful for the early identification of fatal outcomes in COVID-19 patients. More studies are necessary to elucidate the exact mechanism which SARS-CoV- 2 can increase the intestinal permeability in some patients

533. 207. **XBP1 ACTIVATION IN RENAL TUBULAR CELLS MODULATES THE MHC-I ANTIGEN PRESENTATION REGULATING THE PEPTIDE LOADING COMPLEX (PLC)**

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Background: The activation of the unfolded protein response (UPR) under endoplasmic reticulum (ER) stress is closely linked to the pathogenesis of the renal damage. UPR regulates two of the key processes that shape the MHC I peptide processing: protein translation and degradation. We analyze whether XBP1s, a transcription factor key in the UPR activation, impacts on the

MHC I- peptide presentation, and which are their functional consequences. **Methods:** Human tubule epithelial cell line, HK-2, was treated with serum deprivation or hypoxia/reoxygenation or subjected to knockdown of XBP1 by CRISPR/cas9 technology and further analyzed by RNA-seq and HLA class-I LC-MS/MS immunopeptidomics. Additionally, functional T cell analysis was evaluated in these cells lines transfected with the H-2Kb- SIINFEKL-OVA peptide. The binding of XBP1 to target genes was analyzed by ChIP assay. To approach these findings to the clinical practice, spatial-transcriptomics-VISIUM technique was performed in human biopsies from kidney transplanted patients with different clinical outcome (non-rejection, T cell–mediated acute rejection, and IFTA). **Results:** RNA-seq data revealed that the pathway related to MHC-class I antigen presentation triggered under ER stress was mainly XBP1-dependent. XBP1 activation with different damage insults produces changes in the PCL complex inducing the transcription of SEC61A1 and TAPBR. MS- based immunopeptidomic demonstrated that XBP1 deficiency affect differentially to the HLA ligands-repertoire and the absence of XBP1 modifies the recognition of T cells (SIINFEKL- peptide experiments). Furthermore, we observed that XBP1 and PLC genes are upregulated in the tubular compartment of human biopsies associated with a higher degree of rejection and inflammation. **Conclusion:** Our results demonstrate that XBP1 activation under ER stress impairs MHC I-peptide presentation, modifying the expression of PCL molecules and consequently, the antigen recognition. So, XBP1 might be relevant to modulate the T cells-mediated immune response in different clinical context.

534. 265. **THE SYNERGISTIC IMPACT OF TRYPANOSOMA CRUZI INFECTION AND ATHEROSCLEROSIS ON VASCULAR PATHOLOGY: IMPLICATIONS FOR PATIENT-CENTERED PRECISION MEDICINE**

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Previously, we reported that *Trypanosoma cruzi*, the parasite causing Chagas disease, induces pathological vascular remodeling in healthy aortas during both acute and chronic infection. However, its effects on arteries with pre-existing vascular alterations, such as those caused by atherosclerosis, are less understood. Atherosclerosis and T. cruzi infection exert distinct effects on vascular tissues, both marked by chronic inflammation and cellular alterations consistent with transdifferentiation. Additionally, during atherosclerosis, smooth muscle cells (SMCs) dedifferentiate, leading to decreased alfa-SMA expression, increased proliferation, and destabilization of the arterial wall. In contrast, *T. cruzi* infection promotes SMC differentiation, increasing alfa-SMA expression and arterial stiffening. To investigate the potential implications of these two conditions for vascular health, we infected C57BL/6 and ApoE-KO mice with 5,000 *T. cruzi* trypomastigotes. ApoE-KO mice were divided into young and elderly groups, with the latter group exhibiting advanced atherosclerotic plaque development. Cellular populations within the thoracic and abdominal aorta were analyzed by FACS, using uninfected mice as controls. *T. cruzi* infection significantly increased the frequency of CD45+ cells expressing CD11b and alfa-SMA in elderly ApoE-KO mice. Infection also reduced M1 (CD86+iNOS+) and M2 (CD206+Arg1+) macrophage frequencies in both young and elderly mice while increasing CD206+CD36+ cells in the elderly group. Notably, infection consistently elevated alfa-SMA expression across various SMC populations (total alfa-SMA+, alfa- SMA+CD45+, and alfa-SMA-high-expressing) in all groups, with elderly ApoE-KO infected mice showing the highest levels. This increase was accompanied by decreased expression of Ki67 and Arg1, markers indicating a shift toward a more contractile and less proliferative SMC phenotype. These findings suggest that the interaction between *T. cruzi* infection and atherosclerosis may present challenges in treatment, as therapies for one condition could potentially exacerbate the other. This underscores the importance of adopting a patient-centered precision medicine approach to manage individuals with both atherosclerosis and chronic Chagas disease.

535. 308. **IDENTIFICATION OF PRO- AND ANTI-CYTOKINES IN EXPERIMENTAL EXTRAPARENCHYMAL NEUROCYSTICERCOSIS IN DIFFERENT MOMENTS OF EVALUATION**

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Background: Neurocysticercosis is a pleomorphic disease, and its symptoms depend on the number, location and size of the parasites. The extraparenchymal form of the disease has the higher rates of mortality and disability. The cysts in the cerebrospinal fluid (CSF) at the subarachnoid basal cisterns and ventricles can cause obstruction to CSF flow, hydrocephalus, and raised intracranial pressure. The parasite has several mechanisms to avoid host's immune response, which helps to stay viable for long periods of time. Experimental models have provided a better understanding of the disease's pathophysiology. **Objectives**: This study aimed to evaluate the in-situ concentration of several cytokines one and three months after intracranial infection. **Methods**: We used 30 Wistar rats. Twenty animals were inoculated with 50 cysts of *Taenia crassiceps* as previously described, while ten were used as controls. Half of the animals were euthanized one month post-infection and the other half three months post-infection. The brains were harvested and homogenized for the ELISA-sandwich technique to quantify IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, IFN-gamma, TGF-beta, and TNF-alpha. **Results**: At 1 month, the infected animals had significant higher values of IL-2, IL-4, IL-5, IL6, IL-10, and IL-17, whereas at 3 months, only IL-8 and IL-17 were higher for the infected animals. The other cytokines (IFN-gamma, TGF-beta, and TNF-alpha) did not show significant differences at any time. **Conclusion**: These results suggest an immunological shift during the infection: from an initial pro-inflammatory pattern to a permissive profile, which may be related to the chronicity of the infection. These finds can help develop new therapeutic targets.

536. 334. **IDENTIFICATION OF GENE EXPRESSION BIOMARKERS PREDICTING DISEASE PROGRESSION IN NON-SMALL CELL LUNG CANCER TREATED WITH IMMUNE CHECKPOINT INHIBITORS**

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Background: Lung cancer causes 1.8 million deaths annually and has the highest cancer mortality rate, with 85% of cases being non-small cell lung cancer (NSCLC). Immunotherapy with immune checkpoint inhibitors (ICIs) has shown significant progress, using monoclonal antibodies to modulate crucial immune signals. ICIs shows better outcome compared to standard second-line chemotherapy, but reliable prognostic markers are lacking. **Aim:** To identify immunological markers that can distinguish, patients with NSCLC responders to therapy with ICI from non-responders. **Methods:** Gene expression was analyzed using RNA from paraffin-embedded diagnostic biopsies, focusing on 768 genes related to immunometabolism through the Metabolic Pathways Panel. Clinical data were collected, with tumor progression were classified as non-responders, while patients with partial or complete response and stable disease for 6 months or more were classified as responders. Statistical analyses were conducted using NanoString's nSolver and Rosalind software. **Results:** The study cohort included 48 patients, with squamous cell carcinoma being the predominant histological type (52.1%), 62.5% of whom were male, and 58.3% diagnosed at stage IV. Within the cohort, 62.5% had positive PD-L1 status and only 10.4% had never smoked. Seven genes with differential expression were found between patients with and without ICI progression. Three upregulated genes were found in the group without ICI progression: *FOLR3* (p< 0.001), *TYMS* (p= 0.01), and *NOS2* (p= 0.04); and four downregulated genes: *CD19* (p< 0.001), *FCLR2* (p= 0.006), *PGAM2* (p= 0.01) and *ADH1B* (p= 0.03). **Conclusion:** The identification of genes associated with disease progression provides valuable insights for predicting responses to ICI treatment in NSCLC patients. The differentially expressed genes, such as *FOLR3, TYMS, NOS2, CD19, FCLR2, PGAM2,* and *ADH1B*, are linked to key processes including im-

mune pathways, checkpoint expression, cellular metabolism, and tumor growth. These findings suggest potential biomarkers for guiding personalized immunotherapy strategies and improving clinical outcomes for NSCLC patients.

537. 384. **IMMUNOMODULATORY EFFECT OF SMALL EXTRACELLULAR VESICLES (SEVS) FROM METABOLICALLY REPROGRAMMED MESENCHYMAL STEM/STROMAL CELLS (MSCS) ON MEMORY T-CD4+ CELLS**

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Background: An imbalance between proinflammatory and regulatory T-CD4$^+$ subpopulations is a pathogenic feature of chronic autoimmune/inflammatory diseases, thus, balancing the T-CD4$^+$ cells immune response to self–antigens is an ongoing challenge. The use of Mesenchymal Stem/Stromal Cells (MSCs) as a therapeutic approach have been broadly documented due to their immunomodulatory and differentiation ability. However, clinical outcomes are divergent, and the use of metabolically reprogrammed MSCs towards glycolysis have been proposed to enhance their therapeutic capabilities. One of the mechanisms through which MSCs exert their biological effects relies on secreting small extracellular vesicles (sEVs). Therefore, we investigated whether sEVs from metabolically reprogrammed umbilical cord-derived MSCs (UC-MSC) have an enhanced immunosuppressive effect on T-CD4$^+$ cells. **Methods:** sEVs where isolated by ultracentrifugation from basal UC-MSCs (sEVs) or reprogrammed towards glycolysis (sEVs-Glyco) and were characterized by nanozide and FACS analysis. Memory T-CD4 from PBMC and culture in the presence or absence of sEVs/sEVs-Glyco. The internalization of sEVs/sEVs-Glyco on memory T-CD4 and the phenotype and proliferation of proinflammatory Th1&Th17 and anti-inflammatory Treg cells was evaluated by FACS. The immunomodulatory effect of sEVs/sEVs-Glyco was evaluated in a murine model of delayed-type hypersensitivity (DTH) by FACS analysis of murine-PBMC. **Results:** Both vesicles were able to internalize into memory T-CD4$^+$ cells. Furthermore, we found that sEVs/sEVs-Glyco decrease the percentage of IFNγ and IL17-producing Th1 or Th17 cells, while no effects were observed on the percentage of Treg. Both vesicles conditions shown a tendency to reduce the percentage of Th1 and Th17 cells with increased Treg/Th1 and Treg/Th17 ratios in the DTH murine model. **Conclusion:** These findings suggest that sEVs and sEVs-Glyco can modulate the immune response by modifying the phenotypes of memory T-CD4$^+$ cells. Our ongoing challenge is understanding the mechanisms behind the immunomodulatory abilities of MSCs-derived sEVs to potentiate their anti-inflammatory properties.

538. 406. **REVERSING NK CELL EXHAUSTION IN CERVICAL CANCER: A NOVEL BISPECIFIC KILLER CELL ENGAGER TARGETING B7-H6 AND ICOS**

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Background: NK cells play a critical role in the fight against cancer; however, these cells can become exhausted after prolonged exposure to the tumor microenvironment, thus downregulating cytotoxicity and increasing inhibitory receptors, such as PD-1 and TIGIT. We recently described that putatively exhausted NK cells from cervical cancer patients overexpress costimulatory receptors, particularly ICOS. Additionally, we previously found that B7-H6 is an antigen over-expressed by cervical tumor tissues. This led to the current project: the simultaneous targeting of tumor cells via B7-H6 and exhausted NK cells via agonistic recognition of ICOS, using novel bispecific killer cell engagers (BiKEs) in order to enhance the cytotoxicity of exhausted NK cells. **Objective:** To develop a bispecific killer cell engager aimed at enhancing the cytotoxicity of exhausted NK cells against cervical cancer cells. **Methods:** We developed a BiKE with bispecific recognition of a tumor marker expressed in cervical cancer cells (B7-H6) and a costimulatory molecule of NK cell function (ICOS). The bispecificity and stability of the BiKEs were evaluated by flow cytometry, while their binding capacity to target cells was assessed through immunofluorescence using HeLa and SiHa as tumor targets. Cytotoxicity assays were conducted to assess the BiKE effectiveness in enhancing NK cell cytotoxicity. **Results:** We found our BiKEs to be stable for up to 72 h, with the ability to bind target cells via B7-H6. While the BiKEs alone did not affect tumor cell viability, their addition to purified peripheral NK cells dramatically enhanced cytotoxicity against tumor cells. **Conclusion:** In contrast to traditional checkpoint blockade, here we are administering a positive agonist, but only to NK cells that are spatially close to cervical cancer tumor cells, and only to those exhausted NK cells that express the underutilized co-stimulatory receptor. These results highlight the potential of this novel strategy to revert NK cell exhaustion.

539. 500. **VITAMIN C PROMOTES A STABLE PHENOTYPE AND DEMETHYLATED TSDR FOXP3 OF IN VITRO EXPANDED HUMAN ALLOSPECIFIC INDUCED REGULATORY T CELLS**

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One of the limitations for the use of induced Foxp3+ regulatory T cells (iTregs) in immunotherapy is their phenotypic instability caused by epigenetic modifications in specific loci, including hypermethylation of the Foxp3 TSDR. In this context, vitamin C has been shown to induce demethylation of this region through the activation of Tet enzymes, resulting in a more stable and functional Tregs. We have previously demonstrated that allospecific iTregs can be effectively expanded in vitro maintaining a suppressive phenotype and function. However, long term expanded allo iTregs, displayed an increased TSDR methylation. In this study, we assessed the impact of vitamin C on the generation and expansion of allo-specific iTregs, and analyzed their phenotype, suppressive function and te epigenetic status in the presence of pro-inflammatory cytokines. Allospecific iTregs were obtained from co-cultures between monocyte-derived dendritic cells and naïve T cells. After 3 weeks of expansion with TGFbeta, rapamycin and IL-2, the addition of vitamin C resulted in higher levels of Foxp3 and reduced intracellular production of the inflammatory cytokines IFN-gamma and IL-17, compared to cells untreated iTregs. In addition, the suppressive function iTregs cultured with Vitamin C was higher when compared with control iTegs (70% vs 58% of suppression). Interestingly, these results were unaffected by the addition of pro-inflammatory cytokines (IL-6, TNFalpha, and IL-1beta) to the culture. Moreover, the methylation status of the TSDR region, evaluated by pyrosequencing, showed a significant decrease in vitamin C- treated iTregs compared to control iTregs (30% vs 80%). In conclusion, vitamin C promotes the generation of allospecific iTregs with enhanced phenotypic and functional stability in the presence of proinflammatory cytokines which correlates with an increased demethylation of the TSDR region. These findings suggest that vitamin C treated allospecific iTregs can be considered as excellent candidates for immunotherapy aimed at inducing

long term tolerance in transplanted patients.

540. 540. **INNOVATIVE WOUND HEALING APPROACHES: A COMPARATIVE ANALYSIS OF AMNIOTIC MEMBRANE DRESSINGS (HAM-PE) AND BOVINE COLLAGEN MATRIX (BCM) TREATMENTS FOR LARGE ULCERS**

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This comparative study examined the healing progression of a large ulcer by applying two treatments: lyophilised amniotic membrane dressing (hAMpe) in one area and bovine collagen matrix (BCM) in another. Biopsies were collected from both zones at baseline and after 49 days, analyzed through molecular biology techniques (RT-qPCR) and histological staining. Hematoxylin and Eosin (H&E) staining revealed reepithelialization in the hAMpe-treated area, contrasting with no advancement in the BCM zone. The hAMpe treatment showed a more advanced healing stage, compatible with the remodeling phase, particularly in the deep dermis. Picrosirius Red staining under polarized light indicated a higher ratio of collagen types I to III, reflecting a more mature extracellular matrix (ECM). Moreover, anti-CD34 immunohistochemistry showed enhanced vascularization in the hAMpe area (CD34+% area: 4.949 vs. 10.67,****). The fold increase (FI) of VEGF was significantly greater in hAMpe (6.369) compared to BCM (1.593),**. By day 49, inflammatory cell recruitment decreased during hAMpe treatment, whereas BCM continued to induce inflammation, with reduced expression of MCP-1 (FI: 0.2 vs. 1.6, **), IL-8 (0.6 vs. 6.8,****), and CXCL-10. There was also a decrease in IL-1β following hAMpe treatment, with an increase in COL1A2 expression, and higher COL1A1 FI (1.5 vs. 0.8, ****) compared to BCM. Expression of αSMA, characteristic of myofibroblasts, was elevated in the hAMpe area. Comparisons stated were made by t test, alpha=0,05. Biopsy results aligned with in vitro findings, showing negative modulation of IL-1β and NLRP3 expression in LPS-activated THP-1 cells treated with hAMpe, as well as decreased secretion of IL-6 and IL-8 in primary fibroblasts exposed to hAMpe. These findings support the efficacy and safety of hAMpe for healing complex wounds, boosting transition from the initial inflammatory phase into remodeling.

541. 576. **EVS FROM GLYCOLYTIC MSC PROMOTE THE TRANSITION OF MACROPHAGES TO ANTI-INFLAMMATORY PHENOTYPES**

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Osteoarthritis (OA) is a chronic inflammatory disease for which there is no cure, only palliative treatments are available. In OA there is an exacerbated inflammatory response primarily attributed to the activity of synovial-macrophages (SM). Mesenchymal stem/stromal cells (MSC) have been widely described for their therapeutic potential. However, different strategies are needed to improve their immunomodulatory properties. We show that glycolytic MSC (MSCglyco) and their small extracellular vesicles (EVs) could be appealing for developing new therapies for OA. Objective: To evaluate he immunomodulatory properties of the EVs form MSCglyco on the inflammatory activity of macrophages. MSC were isolated from the umbilical cord of healthy donors. MSC were treated with oligomycin for 24 hours to induce a glycolytic metabolism. EVs were isolated from MSCglyco through ultracentrifugation, quantified by nanoparticle tracking analysis, and characterized by transmission electron microscopy and flow cytometry. Macrophages were isolated either from murine bone marrow (BMDM), human peripheral blood (PBM) from healthy donors, or the synovial membrane of OA patients (SM). EVs were added to the culture media of either type of macrophage, and 24 hours later macrophages

were recovered to evaluate EV internalization, surface marker expression, cytokine secretion, and glycolytic flux. The metabolic reprogramming of MSCs does not alter the phenotype of the released EVs. EVs from MSCglyco increase the expression of CD206 while decreasing MCHII and CD86 in LPS-activated BMDMs and decrease the glycolytic flux of LPS/IFNy-activated PBMs. Moreover, SM isolated from OA patients internalize the EVs from MSCglyco, reducing the expression of HLA-DR and CD86 and the secretion of inflammatory mediators. EVs from metabolically reprogrammed MSC have improved therapeutic properties. We demonstrate that EVs from MSCglyco reduce inflammatory profiles in macrophages thus providing compelling evidence for their enhanced therapeutic properties, offering a promising avenue for the development of novel OA therapies with a focus on immunomodulation.

542. 654. **ATTIL12-T CELL THERAPY FOR SIMULTANEOUSLY DISRUPTING STROMA/TUMORS AND INDUCTION OF ENDOGENOUS TCR-T CELLS**

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The anticancer effectiveness of both adoptive T-cell transfer and interleukin-12 (IL12) therapy has been demonstrated in clinical settings, but these promising results have been tempered by prominent challenges, including cytokine release syndrome (CRS), trapping the infused T cells in stromal tissues, and poor activity against heterogeneous solid tumors. To address these challenges, we created a novel type of IL12, a cell membrane–anchored and tumor-targeted IL12, with which to modify T cells. Surprisingly, this modification transforms the biological roles of both IL12 and CAR-T cells. Using T cells expanded from peripheral blood, we modified T cells (including CAR-T cells, TILs, and TCR-T cells) with a membrane-anchored and cell surface vimentin (CSV)–targeted IL12. Infusion of the modified T cells eliminated inflammatory cytokine such as IL6 induction in peripheral tissue, which often occurs with CAR-T cell infusion or IL12-associated injection, thereby eliminating these treatment-associated CRS in treated mice. Of note, the infused T cells not only did not become trapped in tumor stromal tissues but also broke down the stromal tissues (collagen, fibronectin, and cancer-associated fibroblasts) to engage directly with tumor cells, causing remarkable antitumor efficacy in human and mouse large solid tumor models. Thus, these modified T cells are a dual tumor- and stromal cell–targeted therapy for tumors. The underlying mechanism will also be discussed in this meeting

TRANSPLANTATION

543. 055. **PHARMACOLOGICAL ACTIVATION OF TMEM176B BY SALICYLATE-DERIVATIVE NITROALKENE PROLONGS ALLOGRAFT SURVIVAL THROUGH HO-1 INDUCTION**

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Organ and tissue transplants represent the definitive solution when functional failure occurs. However, the low availability of donors, chronic rejection and side effects related to high doses of immunosuppressants are an unsolved problem in medicine. The challenge is to develop therapeutic strategies that modulate immunoregulatory molecules and trigger long-term immune regulatory mechanisms. Pharmacological inhibition or genetic deletion of the ion-channel Tmem176b promotes the antitumoral immune response and appears as a regulatory player in the context of allotransplantation. 5-(2-nitroethenyl) salicylic-acid (SANA), a salicylate-derivative nitroalkene synthetized in our lab, is an anti-inflammatory and metabolic modulator that successfully completed

phase-I clinical trial for obesity and metabolic-related diseases. Nitroalkenes have been reported as ion channels activators. This work objective is to study whether SANA activates Tmem176b and triggers regulatory immune response that prolong graft survival. Our studies confirm that SANA is an activator of Tmem176b ion channel activity. To test the relevance of this effect, we performed a model of minor mismatched skin allograft (skin of male are grafted to female) in WT or *Tmem176b*$^{-/-}$ mice. Subsequently, mice were treated daily with SANA from day -1 before transplantation to day 15 after surgery. Graft survival and the immune infiltrate were analyzed at the skin graft and draining lymph nodes. Our results show that SANA prolongs allograft survival through Tmem176b dependent manner. Mechanistically, the activation of the SANA-Tmem176b axis induces the HO-1 expression in dendritic cells (DCs) both *in vitro* and *in vivo* being dependent on AKT phosphorylation. Downstream, HO-1 promotes the $CD4^{+}FoxP3^{+}$Tregs within the skin graft of SANA treated mice and SANA enhances the effect *in vivo* of Fab anti-CD3 in terms of allograft survival. In summary, the pharmacological activation of Tmem176b linked to HO-1 induction leads to an immunoregulatory pathway that prolongs skin graft survival and improves the effects of systemic immunosuppression.

544. 211. **IMMUNOSUPPRESSIVE TREATMENT AFFECTS THE DYNAMICS OF DONOR-RECIPIENT LYMPHOCYTE TURNOVER IN AN ACUTE CELLULAR REJECTION MODEL OF INTESTINAL TRANSPLANTATION**

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Graft rejection is a major threat in Intestinal transplantation (ITx). Peyer's Patches (PP) participate in the early response that triggers acute cellular rejection (ACR). Immunosuppressants prevent ACR but have a negative impact in the long term. Furthermore, Goblet cells (GC) secrete mucus to the intestinal lumen, providing mucosal protection, therefore, their depletion increases microbial translocation risk. Allogeneic heterotopic ITx procedures were performed using Sprague Dawley rats as donors and Wistar-GFP+ as recipients, divided in no immunosuppressant treatment (n-IS group, n=5), and immunosuppressive therapy receiving tacrolimus 0.6 mg/kg/day administered subcutaneously for 7 days (TAC group, n=6). Graft samples were collected at 0, 4, 7, and 10 postoperative days (POD) for the n-IS group, and at 0, 4, 7, 14 and 21 POD for the TAC group. Histopathological diagnosis of ACR was conducted using the Wu score. Flow cytometry assays were employed to determine the frequency of recipient CD3+, CD4+ and CD8+ in the grafts. Alcian Blue staining was used to count GC in graft samples. TAC group developed severe ACR at 21 POD ($p<0.0001$), whereas in the n-IS group it occurred between 7 and 10 POD. Furthermore, a significant correlation was observed between rejection severity and GC loss ($p<0.0001$). The analysis of recipient lymphocyte showed an early turnover of CD4+ and CD8+ populations in PP compartment, with 25 to 50% lymphocytes being of recipient origin since 4 POD. Moreover, in the *lamina propria* (LP) compartment, the TAC group exhibited delayed recipient CD4+ and CD8+ turnover kinetics until 21 POD. Recipient cell turnover in lamina propria is concordant with the progression of the ACR process. Our results suggest that Tacrolimus can delay turnover kinetics of T-cell compartment in the *lamina propria* without affecting turnover in PP compartment. Pro-homeostatic cells, such as Goblet cells, decrease during the progression of acute cellular rejection.

545. 272. **STANDARDIZATION OF AN ASSAY TO EVALUATE IN VITRO ANTIGEN-SPECIFIC ACTIVATION OF PERIPHERAL BLOOD B LYMPHOCYTE SUBPOPULATIONS OF IMMUNOSUPPRESSED PATIENTS**

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Introduction: Infectious diseases are among the main causes of morbidity and mortality in elderly and immunosuppressed patients. During the COVID-19 pandemic, several studies have pointed to a high mortality rate among this population. This emphasized the need to better understand the mechanisms involved in the generation of immune responses in immunosuppressed patients, as well as to ascertain the longevity of the antigen-specific responses mediated by B lymphocytes. **Objective:** We aimed to standardize an assay evaluating the four main subpopulations of B lymphocytes (switched, unswitched, naive and double negative), and their activation through the expression of CD80 and CD86 after in vitro stimulation with SARS-CoV-2 peptides, with calculation of frequency and absolute number of these subpopulations. **Methodology:** Healthy and immunosuppressed (kidney transplant recipients) individuals were recruited and peripheral blood mononuclear cells were separated before and 30 days after the BTN162b2 vaccine booster dose. For in vitro stimulation, the cells were thawed, left to rest for 24 hours, and then plated at a concentration of 1 million cells per well, then stimulated with a pool of peptides from the Spike, Nucleocapsid and Membrane proteins of the SARS-CoV-2 virus. Stimulation kinetics was carried out to choose the best time and concentration of viral peptides to find the activation levels in the tested individuals. The flow cytometry data were analyzed using Flow-Jo software. **Results and Conclusion:** The results obtained after 4 days of culture with a concentration of 1 ug of each peptide were the optimum time and concentration for carrying out the test. The increase in CD80 and CD86 expression induced with the peptide pool exposure reached values of up to 10%, a strong signal for antigen-specific responses, and unlike non-specific, mitogenic responses, which typically induced increases of up to 40% in the expression of the same molecules.

546. 279. **EVALUATION OF GELMA HYDROGELS IMMUNE RESPONSE AS BIOMATERIAL FOR THE DEVELOPMENT OF FUNCTIONAL IMPLANTS**

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The design of scaffolds/implants to promote immunomodulation in a local and controlled manner is key for tissue engineering and vaccine development. However, it is necessary to comprehend the material at the molecular level to determine how changes in its environment could affect its structure and thus its biological response. Gelatin methacryloyl (GelMA) stands as a suitable material for the development of these scaffolds, where its structure can be tuned by changing different parameters, such as partially promoting collagen native triple- helix configuration prior to photocrosslinking. Nevertheless, how triple-helix configuration can affect the hydrogel biological response has not been extensively studied, nor how these hydrogels can affect immunity. Due to the important role of Dendritic cells (DCs) as antigen presenting cells, our objective is to determine the effect of triple-helix formation on GelMA- hydrogels structuring, and its consequence on DCs phenotype. We produced GelMAs with 2 degrees of substitution (DS) from gelatins from 2 different origins. Samples were incubated at 4°C (triple-helix promotion) or 37°C, and photocrosslinked with UV-light. Hydrogels were characterized and murine splenic CD11c+ DCs were cultured with hydrogels and characterized by flow-cytometry and ELISA. DCs infiltration on implants and draining lymph nodes (dLN) was evaluated by intravenous injection (a day before surgery) of congenically marked CD45.1+DCs into RAG1-KO mice. Results showed that triple-helix formation affected the hydrogel structures displaying an increase in stiffness and overall density but did not exert significant changes on DCs phenotype; however, a trend towards greater activation, given by increased expression of CD86 on DCs and IL-6 release *in vitro*, was observed in the presence of hydrogels with lower DS. Additionally, DCs could infiltrate hydrogels and migrate into dLN with no phenotypic differences regardless of triple-helix formation. These results suggest that these hydrogels and their tunable structure, apparently, do not affect DCs phenotype *in vivo.*

547. 412. **INVESTIGATION OF THE ROLE OF THE XPC GENE IN INFLAMMATION ASSOCIATED WITH EXPERIMENTAL SKIN TRANSPLANTATION**

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Introduction: Various types of injury can cause damage to multiple tissues, especially the skin, which is the most exposed organ in the human being and its preservation is extremely important. In cases of critical burns, allogeneic skin transplantation can be used as a palliative therapy. It is known that the NER pathway is extremely important for recognising spatial distortions in DNA, correcting lesions caused by interactions with ultraviolet radiation, oxidative products and derivatives of polycyclic aromatic hydrocarbons. The absence of XPC interaction with the rest of the hHR23B, TFIIH and Centrin 2 protein complex impairs the initiation of the lesion recognition process, causing inefficiency in the repair process. The phenotypic and functional characterisation of the inflammatory infiltrate and its populations present in the skin graft will be evaluated by comparing the absence and presence of the nucleotide excision repair (NER) mechanism influenced by the XPC gene, using wild-type mice and XPC knockout mice. **Objective**:For this reason, the objective of this work is to evaluate the role of the absence of the XPC gene in allogeneic transplantation and its modulation in immunological responses, evaluating the characteristics of the inflammatory process in the absence of the gene in the global genome repair system (GG -NER) and transcription coupling repair (TC-NER) and CG-NER pathway, where consequently there will be no adequate recognition of lesions in DNA regions. The hypothesis of this project is that due to the absence of the XPC gene, transplant collection will be greater. **Methods**:Male mice of the C57BL/6 and XPCKO strains underwent skin transplantation using Balb/c mice as donors. Graft rejection was monitored by gradually observing the expulsion of the transplanted tissue. Subsequently, samples of the grafted skin, adjacent tissues, draining lymph nodes and spleen were collected for analyses. Immunophenotyping of graft-derived cells, RT-qPCR and flow cytometry were carried out to assess the inflammatory and systemic responses between the groups, comparing the baseline state (control) with three post-transplant stages, according to inflammatory references described in the literature. **Results**: It is concluded that, in the absence of the XPC gene, the survival time of an allogeneic skin graft is significantly reduced compared to individuals who have the gene active. This suggests a possible imbalance in the immune cells, which may result in metabolic and functional alterations. These alterations trigger an exacerbated immune response, leading to rapid rejection of the graft.

548. 485. WHAT WE HAVE LEARNT ABOUT HAEMATOPOIETIC CELL TRANSPLANTATION IN WISKOTT-ALDRICH SYNDROME: CLINICAL FEATURES AND IMMUNE RECONSTITUTION

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Background: Haematopoietic cell transplantation (HCT) is a curative treatment for Wiskott-Aldrich syndrome (WAS). Post-HCT clinical features and immune reconstitution depend on varied factors. **Objective:** Describe the outcome of a cohort of post-HCT WAS patients. **Methods:** Retrospective data collection from 5 clinical records from post-HCT WAS patients. **Results:** 5 male patients diagnosed with WAS. Median follow-up: 5.6 years [1-12], median age at transplant 21 months [14-37]. All received BCG vaccine. Pre-HCT patients presented: 3 (60%) severe infections, 3 (60%) CMV viremia, 2 (40%) growth failure, and 1 (20%) BCGitis. Patients received peripheral blood stem cells (n=3) or cord (n=2) from mismatched (n=2) or matched (n=2) unrelated or haploidentical (n=1) donors using 2 (40%) busulfan-cyclophosphamide conditioning, 2 (40%) busulfan-fludarabine and 1 (20%) fludarabine-cyclophosphamide. All received ATG. Tacrolimus as GvHD prophylaxis in 5 (100%), 3 (60%) associated with methotrexate. 4 patients (80%) developed acute, and 4 (80%) chronic GvHD. 4 (80%) patients had CMV and 1 (20%) EBV viremia, and 2 (40%) VOD. During the follow-up: 4 (80%) had severe infections and 1 (20%) BCGosis, 3 (60%) developed lung disease, 2 (40%) required high immunosuppression treatment, 2 (40%) improved growth, 2 (40%) had musculoskeletal problems and 1 (20%) autoimmunity. Post-HCT evaluations showed ophthalmology (80%), cardiology (40%) and dermatology (40%) complications. 4 patients had donor chimerism performed>70% [77-100]. All presented cellular immune reconstitution between day +180 and +540 (measured as Naive LTCD4+) [19-61%]. Only 1 of 3 patients showed

full humoral reconstitution: normal immunoglobulins levels, vaccine response and B-cell subsets, while the other 2 had a partial response. Humoral evaluation has not been performed in 2 patients. **Conclusion:** All the patients developed acute or chronic GvHD, most had viremia or required high doses of immunosuppression. These are factors related to late immune reconstitution and correlated to what happened with our cohort.

549. 539. **HLA GENETIC FREQUENCY IN RENAL TRANSPLANTATION PATIENTS, DEAD BY COVID-19**

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Background: HLA association with disease has been widely reported, as well as the capability of some viruses and tumors to use different strategies for downregulating HLA expression to escape the recognition from T lymphocytes. **Objective:** To analyze probable genetic associations of the HLA loci in patients with a functioning kidney transplant who died of COVID-19. **Method**: We studied 60 adult patients from the kidney transplant center of CUCAIBA, Buenos Aires, Argentina, who had a functioning kidney transplant and died of COVID-19. HLA genes was done by molecular biology SSP or SSO, and included genes from HLA Class I (A and B) and Class II (DRB1). For comparison with the healthy Argentine control population. We included 2657 people from all over the country from the INCUCAI CPH National Donor Registry. **Results**: Here we show the genetic analysis of each gene mentioned, comparing the frequency, appearance or absence of each HLA A, B and DRB1 genes determined in both populations. The percentages obtained, presented as patient (p), control (c) and variation (v), are shown below: HLA A23: 0.8% p, 3% c, -73%v; HLA A31: 15.8% p, 7.8%, c +102% v; HLA B57: 4.2%,
2.4% c, +75% v; HLA B39: 10% p, 6% c, +67% v; HLA B27: 0.8% p, 2.2% c, -64% v y HLA
DR8: 12% p, 8.1% c, +48% v. **Conclusion**: We observed a positive variation for some genes like HLA A31, B57, B39 and DR8, these genes would predispose to the disease outcome, on the other hand we observed, a negative variation for some genes like HLA A23 and B27 respect the frequency observed in a healthy population, these genes would we able to protect the population of kidney transplant patients who didn't die from COVID-19.

550. 634. **FIBRIN HYDROGEL: A 3D PLATFORM FOR IMMUNOLOGICAL STUDIES IN RENAL TRANSPLANTATION**

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Peripheral blood and 2D cell culture models are commonly used to study immune cell parameters, yet they fail to accurately represent tissue-resident cells involved in autoimmune diseases or organ transplantation. The aim of the present study was to generate and validate 3D fibrin hydrogels (FH) as a model to study immune cell parameters inside the tissue. For this purpose, peripheral blood mononuclear cells (PBMCs) were isolated by a Ficoll Hypaque gradient from healthy subjects (HS) and kidney transplant immunosuppressed patients (KTIP). After FH was constructed (RPMI 1640, 40% plasma, and 0.1% $CaCl_2$), PBMCs were seeded on top of it. On the 2nd day, the non- and gel-migrated cells were recovered from the supernatant and the gel, respectively. Cell immunophenotyping was evaluated by flow cytometry. The mean percentages of positive cells for each marker present in supernatants and in the gels were compared, between HS and KTIP (Mann Whitney test) and among themselves (Wilcoxon matched-pairs signed rank test). In HS, the $CD4^+$ and $CD8^+$ cells are distributed equally in the supernatant and the FH. The same was observed for $CD8^+$ cells derived from KTIP. However, there were fewer $CD4^+$ cells in the FH than in the supernatants when cells were derived from KTIP ($p<0.01$). Thus, the CD4/CD8 index was lower for KTP than HS. No differences were found between the levels of CD40L expression in T cells present in supernatant or gel, but a positive correlation was found between these and the percentage of $CD19^+$ $CD40^+$ lymphocytes present in PBMC from KTIP (Spearman non-parametric correlation, $r=0.9$, $p= 0.0417$). We conclude that the culture of PBMCs in 3D FH may be a better model to evaluate immune cell parameters in KTIP; perhaps reflecting the immune status of immunosuppression of these patients.

VACCINES

551. 020. A CHLAMYDIA MURIDARUM MAJOR OUTER MEMBRANE PROTEIN NANOVACCINE ADJUVANTED WITH ESCHERICHIA COLI DOUBLE MUTANT LABILE TOXIN BOOSTED IMMUNE RESPONSES AND PROTECTIVE EFFICACY AGAINST A GENITAL CHALLENGE

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Chlamydia trachomatis (Ct) is the most frequent sexually transmitted infection worldwide. Ct infections often lead to reproductive morbidities in women and re-infections, thus making it a major public health concern. To date, there is no licensed vaccine for Ct. In the present study, we developed a chlamydial nanovaccine by encapsulating *Chlamydia muridarum* (Cm) recombinant major outer membrane protein (MOMP) in PLGA (poly (D, L-lactide-co-glycolide) nanoparticles admixed with the *Escherichia coli* **d**ouble **m**utant **L**abile **T**oxin (dmLT) mucosal adjuvant (named dmLT-nV). We hypothesized that dmLT would potentiate MOMP-induced immune responses and enhance the protective efficacy of the nanovaccine in immunized mice. Our hypothesis was tested by *in vitro* and *in vivo* studies. We observed that dmLT boosted the MOMP-specific transcriptional expression of the TLR-2 pathogen recognition receptor and CD80 co-stimulatory molecule of mouse J774 macrophages and the production of Th1 cytokines (IL-6, IL-12p40). Mice received three subcutaneous immunizations with dmLT-nv and then were challenged vaginally with Cm (10^5 IFU (inclusion forming units) to enumerate chlamydial burden over time. Our results revealed boosted protection of dmLT-nV-immunized mice after challenge, as evidenced by a lower chlamydia burden than nanovaccine alone. Furthermore, dmLT-nV immunized-protected mice produced elevated MOMP-specific serum IgG2a and IgG2b (Th1) compared to IgG1 (Th2) antibodies, indicating a heightened Th1 response. Restimulation of T-cells from dmLT-nV immunized and challenged mice revealed enhanced MOMP-specific IFN-γ and IL-17 cytokines, CD4+ proliferating cells, and intracellular IFN-γ+ producing CD4+ T cells, including memory (CD44+CD62L+), and effector (CD44+CD62L-) phenotypes. Overall, dmLT boosted MOMP-induced immune responses and the protective efficacy of the chlamydial nanovaccine against a genital challenge in mice. Our results are significant in developing a chlamydial vaccine for preclinical translational research, a necessary step to significantly impact global public health and reduce the burden of chlamydial infections and related reproductive morbidities.

552. 043. MAPPING LINEAR ANTIBODY EPITOPES IN COVID-19 INFECTION

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Early 2020 witnessed an outbreak of COVID-19,transmitted by SARS-CoV-2.The illness infected more than 700 million people and 7 million deaths, said by Worldometer. Nowadays,it is clear that the extreme situation experienced was only overcome thanks to the rapid production and application of vaccines.In spite of it, the distribution of immunizations happened unevenly. Until 2023, the total population vaccinated with at least one dose of a COVID-19 was 82% in the USA and 5% in Haiti,according to the United Nations. It illustrates how the economy influences public health. These vaccines are based on high cost techniques,which represents a challenge for low-income countries. An alternative is creation of peptide-based vaccines. My project investigates the presence of linear B lymphocyte epitopes of the proteins expressed in COVID-19,characterized as vaccine targets. For this purpose, we performed an *in silico* analysis of a peptide microarray covering the expressed sequences of the viral genome. From serum of asymptomatic infected patients, we verified the presence of linear peptide epitopes immunodominant for induction of protective antibodies. We tested a cohort of hospitalized patients. Some of them improved and were discharged and others died. These recovereds' serum demonstrated more interaction with pan coronaviruses. Furthermore, we developed protein dynamics observations and the survivor's samples are more rich in epitopes from Spike protein. In conclusion, those who possessed more epitopes to coronaviruses demonstrated efficient immune response. As a perspective, we produce peptide containing the sequence of epitopes that are more recognized on the array. Our group is validating the capability of it to stimulate the antibodies production on

serum of infected patients. Through the ANOVA method we analyzed the results.Such approaches are expected to be able to provide a set of selected epitopes useful in guiding experimental efforts to develop vaccines against SARS-CoV-2.

553. 048. **IMMUNE RESPONSE INDUCED BY PLASMIDS THAT CODIFY D-S1N AND O-SN SARS-COV-2 FUSION PROTEINS IN A PRECLINICAL MODEL**

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1. CINVESTAV

Background: The Next Generation of SARS-CoV-2 vaccines is focused on the evaluation of antigens different to spike protein to induce a long-lasting immune response and deal with the different Variants of Concern (VOC) of this virus. Nucleocapsid SARS-CoV-2 protein is highly conserved among variants and coronaviruses. Also, induces a good cellular immune response. Preclinical studies showed that the combination of spike and nucleocapsid protein induces a higher immune response and the capacity to deal with different variants of SARS-CoV-2. **Objective:** Generate plasmids that codify for two fusion proteins of SARS-CoV-2 based on Delta and Omicron strains and evaluate the immune response induced in a murine model. **Methods:** By in silico approaches, generate two proteins that include the most immunogenic regions of Spike (S) and Nucleocapsid (N) proteins. The sequences of these proteins were cloned in pcDNA3.1 and named pcDNA3.1/D- S1N and pcDNA3.1/O-SN. Immunofluorescence evaluates the expression and the identity of the fusion proteins. BALB /c mice were immunized with pcDNA3.1/D-S1N and pcDNA3.1/O-SN. Three doses of DNA were administrated at intervals of 20 days. After immunization, bleedings were performed, and serum samples were obtained. The mice were euthanized 67 days after priming, and the spleen's cellular response was analyzed. **Results:** Specific antibody responses of IgM and IgG against N and S1 proteins from SARS-CoV-2 were observed in immunized mice. Neutralization activity was observed too. Production of IFN-γ was observed in T CD4+ and CD8+ cells from the spleen. Additionally, cross-reaction of sera with different RBD VOCs was detected. **Conclusion:** DNA immunization with pcDNA3.1/D-S1N and pcDNA3.1/O-SN is a suitable strategy to induce a specific humoral and cellular immune response against SARS-CoV-2.

554. 053. **SILVER NANOPARTICLES OBTAINED BY MYCOSYNTHESIS AS POTENTIAL VACCINE ADJUVANTS**

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Subunit vaccines are currently considered the best class of vaccines in terms of biosafety. However, they need adjuvants to be effective, with few approved for clinical use. Silver nanoparticles (AgNPs) enhance vaccine efficacy by providing immunogenicity to protein antigens. AgNPs, formed by the reduction of silver cations, can be synthesized biologically in a simple, fast, cost-effective, and eco-friendly manner. Fungal-mediated synthesis (mycosynthesis) offers biotechnological benefits, such as colloidal stabilization through the formation of a biomolecular capping, whose composition varies with the fungal species and its growth medium. Herein, nine AgNPs were synthesized using the fungi *Phanerochaete chrysosporium*, *Penicillium expansum* and *Punctularia atropurpurascens*, each one grown in three different culture media (PDB, MGYP and MEB). Physicochemical characterizations of the obtained AgNPs were performed, and their biocompatibility profiles were assessed both in vitro and in vivo. Finally, their adjuvant activities were evaluated in mice immunized with ovalbumin (OVA) as a model protein antigen. Synthesized AgNPs were all spherical, with sizes in the range 7-78 nm, Z potential values of -16.9 to -22.9 mV, and capping-protein content of 4-125 fg/nanoparticle. Additionally, AgNPs showed good in vitro biocompatibility profiles, exhibiting values for HA_{50} from $6.5x10^7$ to values $>1.0x10^9$ nanoparticles/mL, and CA_{50} in the range $3.7x10^7$-$1.0x10^9$ nanoparticles/mL; as well as absence of acute toxicity effects in vivo. Finally, mice were immunized twice with a mixture of OVA and AgNPs, and serum titers of OVA-specific antibodies were determined by ELISA. In this sense, four AgNPs showed significant adjuvant activity at the tested

dose, enhancing the OVA-specific IgG response by 10-fold in comparison to the negative control (OVA in saline). In particular, AgNPs obtained from *P. expansum* grown in PDB showed an outstanding IgG2a-polarizing activity. In summary, the preliminary results reported here support the development of novel vaccines adjuvants based on AgNPs obtained by mycosynthesis.

555. 060. **DENDRITIC CELL TARGETING USING A DNA MULTIEPITOPE VACCINE AGAINST EGFR FOR ACTIVE BREAST CANCER IMMUNOTHERAPY**

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Purpose: DNA vaccines are a promising approach to cancer treatment. Active specific immunotherapy is a pivotal strategy to treat EGFR-positive tumor cells. EGFR overexpression appears to be associated with reduced overall survival and disease-free survival in breast cancer patients. The low immunogenicity of DNA vaccines in humans can be overcome by optimizing constructs. **Methods:** For this reason, we aimed to generate a highly optimized DC- targeting DNA multiepitope breast cancer vaccine against the overexpressed target EGFR. In silico construction included several CTL, CD4+ and CD8+ T lymphocytes epitopes. To enhance immunogenicity and increasing antigen presentation, sequences of a single chain Fv antibody specific for the DC endocytic receptor DEC205 were attached with a linker to the N-terminal of the EGFR epitopes . BALB/c mice were intramuscularly immunized three times with 100ug of plasmid DNA encoding either scFvDEC205-EGFR; after first dose they were challenged with murine mammary adenocarcinoma 4T1 cells. Immune response were assessed. The results obtained were analyzed using GraphPad Prism 5 program. **Results:** Overall, the results suggest therapeutic potential for scFvDEC205-EGFR DNA vaccine against triple negative breast cancer. The vaccine constructs was sequenced and showed 100% identity in the nucleotide sequence aligned with the murine EGFR cDNA. This vaccine displayed the capacity to control primary tumor development, increase DC frequency and expression of MHC II molecules after specific-stimulation with EGFR peptide pool, decrease systemic levels of TNFα, and increase frequency of TIL. **Conclusion:** In this research, a new active immunotherapy strategy for triple negative breast cancer treatment were constructed by linking specific EGFR protein epitopes for helper and cytotoxic T lymphocytes to sequences of a scFv antibody targeting DC endocytic receptor DEC205. Its capacity to modulate immune responses in vivo indicating potential efficacy. Further studies are recommended to study the scDEC205-EGFR DNA vaccine effect in solid tumors overexppressing EGFR.

556. 083. **ASP-2/TRANS-SIALIDASE CHIMERIC PROTEIN INDUCES ROBUST PROTECTIVE IMMUNITY IN EXPERIMENTAL MODELS OF CHAGAS DISEASE**

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Immunization with Amastigote Surface Protein-2 (ASP-2) and Trans-sialidase (TS) antigens, whether delivered as recombinant proteins, plasmid-encoded, or via human adenovirus 5 (hAd5), provides robust protection against various lineages of *Trypanosoma cruzi*. In this study, we developed a chimeric protein combining the most immunogenic regions of TS and ASP-2 (TRASP) and compared its immunogenicity with our standard heterologous prime-boost protocol using plasmids and hAd5. Mice immunized with the TRASP protein associated with Poly- ICLC (Hiltonol) exhibited strong resistance to *T. cruzi* challenge, showing a 90% reduction in parasitemia and 100% survival. This protection persisted for at least three months after the last boost and was comparable to the protection achieved with the DNA/hAd5 protocol. TRASP induced high levels of T. cruzi-specific antibodies and IFN-γ-producing T cells, with protection primarily mediated by $CD8^+$ T cells and IFN-γ. We also assessed the toxicity, immunogenicity, and efficacy of TRASP and DNA/hAd5 formulations in dogs. Mild side effects were observed at the vaccine injection site, but these resolved within 72 hours. The chimeric protein with Poly-ICLC elicited high levels of antibodies and strong $CD4^+$ and $CD8^+$ T cell responses, whereas the DNA/hAd5 approach induced no antibodies but a robust $CD8^+$ T cell response. Both vaccines protected dogs from T. cruzi challenge, evidenced by a 93% reduction in parasite load in the heart and decreased in-

flammatory infiltrate. Despite similar efficacy, we conclude that TRASP associated with Hiltonol is preferable to the DNA/hAd5 vaccine. This is due to pre-existing immunity against the adenovirus vector and the overall cost-effectiveness for development and large-scale production.

557. 086. **EPIDEMIOLOGICAL ANALYSIS OF ACUTE FLACCID PARALYSIS IN BRAZIL BETWEEN 2011 AND 2021**

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Background: The non-eradication of wild poliovirus worldwide brings a risk of virus reintroduction in Brazil. It is crucial to maintain high vaccination coverage in children, along with sensitive surveillance of Acute Flaccid Paralysis (AFP). The Brazilian Ministry of Health has risk communication mechanisms including the immediate notification and investigation of AFP cases in children under 15 years old and individuals of any age with a history of travel to countries with poliovirus. Objectives: This study aimed to describe the epidemiological profile of AFP in Brazil between 2011 and 2021. Methods: This retrospective and cross-sectional epidemiological study evaluated the criteria of age group, race, gender, final classification and case evolution from 2011 to 2021. Data were obtained from the Department of Informatics of the Unified Health System (DATASUS), accessed through the Health Information Tabulator (TABNET). Results: The country recorded 4,464 cases of AFP, with an average incidence of 423.9 cases per year. The most frequent age group was 1 to 4 years (1,667 cases), followed by 10 to 14 years (1,382 cases), and 5 to 9 years (1,371 cases), successively. By race, the brown population was the most affected, 2,328 cases. By gender, 2,600 cases were male, 2,063 were female. By final classification, there were 3 compatible cases, 6 vaccine- associated, 99 inconclusive, and 3,936 discarded cases. There were no records in the category “confirmed poliovirus”. AFP evolution resulted in recovery with sequelae in 992 cases, recovery without sequelae in 2,539 cases, deaths from other causes in 83 cases. Conclusion: Despite Brazil’s certification as free of wild poliovirus, active surveillance and the maintenance of high vaccination coverage are essential to prevent this virus reintroduction. The large number of AFP cases and the small number of that total being associated with the poliovirus vaccine highlights the ongoing importance of effective monitoring and vaccination.

558. 089. **GLP PRODUCTION OF A PLASMODIUM VIVAX RIBOSOMAL PROTEIN FOR THE FORMULATION OF UNIVERSAL MALARIA VACCINE**

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Malaria remains a devastating infectious disease, causing thousands of deaths annually. *Plasmodium vivax* is the predominant species in Latin America and the second most common globally, responsible for recurrent outbreaks that hinder eradication efforts. Currently, only two WHO-approved vaccines target the pre-erythrocytic stage of *Plasmodium falciparum*, with limited efficacy against severe disease and child mortality. Consequently, developing an effective vaccine against various *Plasmodium* species is crucial for reducing the disease burden. Our group conducted an immunopeptidomic analysis to identify peptides presented by reticulocytes infected with *P. vivax* (Pv). Most of the eluted peptides were derived from ribosomal proteins, highly conserved across *Plasmodium* stages and species. We elected one vaccine candidate to proceed with Good Laboratory Practice (GLP) production for analytical characterization, preclinical safety studies, and vaccine formulation. Using a non-lethal *P. yoelii* mouse malaria model, we evaluated cross-species protection. We demonstrated that cytotoxic T cells specific to Pv antigens (Ag-CTLs) directly lyse reticulocytes infected with *P. yoelii*. Adoptive transfer of these Ag-CTLs significantly reduced blood parasitic load. Immunization with the L30 protein induced a protective response against P. yoelii challenge, activating CD4+ and CD8+ T cells, specific humoral responses (IgG and IgG2c), and expansion of effector and central memory T cells. These preclinical results suggest that the ribosomal protein L30 could be a potential universal malaria vaccine candidate. For GLP batch production, we produced a master cell bank with optimal L30 protein expression. We enhanced batch yield, protein stability in the final buffer, and removal of endotoxins and host cell proteins. Additionally, we performed characterization and validation tests of the L30 protein

and compared adjuvants for the vaccine formulation. The results were promising, and future steps include developing stability assays, dose and adjuvant formulation, and vaccine packaging to proceed to clinical trial Phase I/II.

559. 096. **EXTENDED IDENTIFICATION OF ENZYMATIC PEPTIDES IN A REPRESENTATIVE BITIS ARIETANS SNAKE VENOM SAMPLE: A NEW MODEL OF COMPLEMENTARY THERAPY FOR SNAKEBITE**

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Snakebite is a significant global public health concern, impacting individuals worldwide, particularly in tropical and subtropical regions of Asia, Africa, Oceania, and Latin America. These areas experience high morbidity and mortality rates due to venomous snakebites, with sub-Saharan Africa, South and Southeast Asia, Papua New Guinea, and Latin America being disproportionately affected. In Africa alone, snakebite-related deaths range from 3,500 to 32,000 annually, and the difficulty in treatment of envenomation generates high numbers of illnesses and disabilities. This study focuses on identifying enzymatic activities within peptides derived from *Viperidae* snake venoms using mass spectrometry and later developing specific monoclonal antibodies targeting these enzymes. Mass spectrometry analysis revealed 1099 distinct proteins in SDS-PAGE bands, demonstrating significant amino acid sequence conservation similar to proteins found in other viperid snakes. The discovery of shared protein similarities between *Bitis arietans* venom and other snake species suggests the potential for developing novel complementary therapies utilizing anti-toxin antibodies. Furthermore, developing Complementarity-Determining Regions (CDR's) within these monoclonal antibodies is anticipated to enhance toxin neutralization efficacy and improve treatment outcomes for diverse snakebite-induced symptoms. This research of our understanding of the biochemical properties of snake venoms and offers promising avenues for developing more targeted and efficacious therapies for snakebite management. By leveraging molecular insights and antibody technology, this study aims to mitigate the impact of snake envenomation globally, particularly in regions where snakebite incidents pose a significant public health burden.

560. 097. **EFFECT OF DIFFERENT PLATFORMS OF ORAL VACCINES AND CONTRIBUTION OF THE VISCERAL ADIPOSE TISSUE FOR MUCOSA VACCINE IMMUNITY**

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Background: 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 towards innocuous antigens. Not only the gut-associated lymphoid tissue, but also other organs contribute to ensure homeostasis in mucosal tissues, such as adjacent visceral adipose tissues (VAT). Adipose compartments, such as mesentery, have already been described as infection-induced memory cell reservoirs, but it is not clear whether these tissues could sustain mucosal vaccine induced memory cells. **Objectives:** Our goal is to elucidate the contribution of VAT, particularly the mesentery and omentum, as major reservoirs of oral vaccine-elicited memory cells. **Methods:** We tested different experimental oral vaccines: oral recombinant Modified Vaccinia Ankara virus expressing ovalbumin (rMVA), sublingual rMVA, oral double mutant heat labile toxin/ovalbumim (dmLT/OVA), and a prime-boost system using sublingual MVA and dmLT/OVA simultaneously. C57BL/6 CD45.1 mice, transferred with OVA-specific CD45.2, were immunized with the respective oral vaccines. We performed flow cytometry and ELISA assay for cell and antibodies characterization, respectively. We applied analysis of variance, T and Tukey test on the data. **Results:** dmLT/OVA induced the highest levels of OVA-specific IgG1 and IgG2a antibodies in the serum and OVA-specific IgA in the intestinal lavage. Both dmLT/OVA

and the prime-boost system were more efficient in promoting the activation of cellular adaptive responses in the gut lamina propria. The homologous vaccines based only in the viral vector presented low mucosal immunogenicity. Notably, dmLT/OVA was the only formulation that elicited OVA-specific cells in the omentum of the immunized mice. **Conclusion:** These results indicate the use of dmLT as an adjuvant for oral vaccines and also suggest the omentum as a reservoir of antigen-specific vaccine activated T cells.

561. 144. **CHALLENGES IN THERAPEUTIC AND PROPHYLACTIC MUCOSAL VACCINE ADJUVANT DEVELOPMENT**

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562. 169. **PRODUCTION AND EVALUATION OF THE IMMUNOGENICITY OF A VACCINE BASED ON THE MVA VIRUS AGAINST MPOX PRODUCTION AND EVALUATION OF THE IMMUNOGENICITY OF A VACCINE BASED ON THE MVA VIRUS AGAINST MPOX**

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In 2022, several cases of Mpox were reported in countries where the disease is not endemic. Some studies suggest that a vaccine based on Modified Vaccinia Ankara (MVA) virus is effective against Mpox. This work aims to optimize and produce a Brazilian vaccine based on the MVA virus and evaluate its in vivo immunogenicity. Balb/C mice were immunized intramuscularly with 1x108 MVA virus using priming and boosting. After 42 days of priming, blood was collected to assess immunogenicity. High titers of total IgG, IgG2a, and IgG1 were observed. Antibodies, post-prime/boost, capable of neutralizing 50% of viral particles, occurred at a dilution of 1/320. Mice immunized and challenged with the Mpox virus survived the infection. However, the unvaccinated and challenged group had 100% mortality. The viral load in challenged animals' lungs, spleen, and liver was assessed by plaque assay and qPCR; in non- immunized and challenged animals, high Mpox titers were observed in these organs. Immunized animals did not present viral load in the evaluated organs. The evaluation of the cellular response in the lungs of the animals showed significant production of CD4+ and CD8+ cells in the immunized animals. In addition, histopathological analyses showed a significant reduction in lung lesions caused by the Mpox virus. IFN, TLR, CD4+, CD8+ knockout animals, and B cells, vaccinated and subsequently challenged, show that the response depends on neutralizing antibodies, and the absence of TLR9 plays a vital role in survival. Our results show that a vaccine based on the MVA virus can provide a protective and sterilizing response when challenged with the Mpox virus.

563. 210. **CHARACTERIZATION OF A NANOPARTICLE-BASED VACCINE AS A BOOSTER FOR COVID-19**

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Nanotechnology has recently emerged as a key player in biomedicine. Our research focuses on polymeric nanoparticles (Np), which we have shown to be effective as delivery systems and adjuvants for systemic vaccines. In response to the COVID-19 pandemic, we aimed to develop a systemic vaccine employing nanoparticles and

SARS-CoV-2 proteins, specifically the receptor binding domain (RBD) of ACE2. We characterized Np as adjuvants by evaluating their internalization in antigen-presenting cells (APC) and activation of CD8+ OT-I lymphocytes *in vitro*, as well as their immunogenicity and protection effects *in vivo*. Balb/c mice were initially vaccinated with two doses of the Pfizer® vaccine, followed by a booster with RBD and either Np, Alum, or Alum+CpG after 28 days. Humoral and cellular immune responses were assessed 15 days after boosting by ELISA and flow cytometry. In another experiment, K18-mice were vaccinated using a heterologous schedule (Pfizer® as the first dose and our vaccine candidates as the second dose). Fifteen days after the second dose, the mice were exposed to Omicron BA.5 and sacrificed 4 days post-infection. Histopathological analyses and viral load measurements were performed on lung and nasal tissue samples. Results indicated that Np were internalized by APC, leading to lysosomal destabilization and facilitating antigen presentation, which subsequently activated T-cells ($p<0.05$). In the boosted mice, serum levels of RBD-specific-IgG were significantly elevated compared to the PBS-boosted-group, along with an increase in IFN-gamma-levels. Interestingly, a lower viral load was found in mice immunized with our vaccine candidates compared to those immunized with PBS ($p<0.05$).
In conclusion, nanoparticles exhibited strong adjuvant properties, inducing T-cell activation *in vitro* and eliciting high levels of immune response when used as a vaccine booster. Furthermore, these nanoparticles provided protection against SARS-CoV-2 *in vivo*. These findings support the potential of Np-based formulations as a promising component of our COVID-19-vaccine candidate.

564. 229. **ISCOM-MATRICES ADJUVANTED INFLUENZA VACCINE: IMMUNE RESPONSE EVALUATION IN AN AGED MICE MODEL**

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Enhancing vaccine efficacy against respiratory viruses is especially crucial for older adults, as they are more susceptible to severe infections due to underlying health conditions and the aging of their immune system. The addition of saponin-based adjuvants is a promising approach to increase the effectiveness of vaccines. In this study we evaluated the use of ISCOM-matrices, a self-assembled immunostimulating complex combining *Quillaja brasiliensis* saponins along with cholesterol, and phospholipids (IMXQB), as an adjuvant for the seasonal trivalent influenza vaccine (TIV) in an aged mice model. Aged female BALB/c and C57BL/6 were divided into 3 groups (n = 10 per group) and immunized twice (on day 0 and 14) subcutaneously with TIV adjuvanted with IMXQB (TIV-IMXQB), TIV, or saline solutions, respectively. Animals were injected in the hind neck with 100 µL of the corresponding vaccine formulation or saline solutions. Blood samples were collected, for all mice, on days 0, 14, and 28, prior to immunization. A month after the first immunization, all animals were challenged with A/Uruguay/897/2018 (H1N1) pdm09-like virus. Mice were monitored daily for 14 days after infection, assessing weight loss and other clinical signs of virus-induced illness, such as ruffled fur and lethargy. Mice were euthanized if they lost more than 20% of their initial body weight or displayed other signs of severe suffering. Statistical significance was assessed by one-way-ANOVA Kruskal–Wallis test with uncorrected Dunn's post- test correction for multiple comparisons compared to the control group (TIV). The adjuvanted vaccine promoted higher titers of IgM, IgG (and isotypes), serum hemagglutination inhibition titers (HAI) and improved recovery rate in both strains of mice after the H1N1pdm09-like virus lethal challenge in comparison to the commercial vaccine. Overall, TIV-IMXQB improved the immunogenicity compared to TIV by enhancing systemic immunity in old mice conferring a faster recovery after the H1N1pdm09-like virus challenge. Therefore, representing a promising platform for next- generation viral vaccines.

565. 241. **CO ENCAPSULATION OF ANTIGENS AND ADJUVANTS IN POLYMERIC NANOPARTICLES FOR THE DEVELOPMENT OF AN ORAL VACCINE FORMULATION**

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Oral vaccines are easy to administer but their development still faces many challenges, such as the hostile gastrointestinal environment.
The protease inhibitor U-Omp19 from *Brucella* spp. was shown to be an oral vaccine adjuvant that protects co-delivered antigens from proteolysis in the gastrointestinal tract and increases antigen specific adaptive immune responses. In this work, we aim to protect vaccine formulation by encapsulating it in poly-(lactic-co- glycolic)-acid (PLGA) nanoparticles. Eudragit, a polymer that dissolves at a pH higher than 7.4 was used to cover these nanoparticles and prevent antigen absorption in the duodenum where the response could be tolerogenic, and to deliver the vaccine to the large intestine where adaptive immune responses can be triggered. Nanoparticles encapsulating OVA or HbsAg as antigens were synthesized using the double emulsion- solvent evaporation (DE-SE) method, in both cases U-Omp19 was used as the adjuvant. Encapsulation efficiency was determined by SDS-PAGE. Characterization was carried out using DLS, SEM and z-potential. HT-29 and Caco-2 cell lines were utilized to evaluate antigen internalization using flow-cytometry. BALB/c mice were orally immunized with the antigen and adjuvant encapsulated in nanoparticles with control groups receiving the non-encapsulated vaccine formulation. Specific antibody responses were determined in stool samples by ELISA. Four weeks after the last immunization adaptive immune responses were evaluated in the Peyer Patches and spleen by flow cytometry. IFN-g was determined in splenocyte cell culture supernatants by ELISA. An increase in the internalization of the encapsulated antigen was observed compared with the non-encapsulated antigen in both cell lines. Mice immunized with nanoparticles (HbsAg+ U- Omp19) showed a significant increase in both specific IgA response, follicular T cell population in the spleen and of B220+ CD19+ IgA+/IgG+ antigen specific cells in the Payer Patches in comparison to the control groups. Mice immunized with nanoparticles (OVA+ U- Omp19), showed a significant increase in IFN-g in splenocyte cell culture supernatants after being stimulated with OVA. In conclusion, we developed polymeric nanoparticles that encapsulates both the antigen and the adjuvant increasing antigen internalization, IgA specific antibodies in feces, an increase in the immune response mediated by B cells in the Payer Patches, and Th1 response.

566. 248. ARTIFICIAL INTELLIGENCE-ASSISTED IDENTIFICATION OF POTENTIAL CORRELATES OF PROTECTION IN A VACCINE AGAINST TRYPANOSOMA CRUZI

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Background: Chagas disease, caused by *Trypanosoma cruzi (T. cruzi)*, remains without a licensed vaccine, and correlates of protection (CoPs) have not been reported. We and others have previously described that the regulatory arm of the immune system, including myeloid-derived suppressor cells CD11b+Gr-1+ (MDSCs), can play an important role during both immunization assays and after *T. cruzi* infection. Depletion of MDSCs with 5- fluorouracil (5FU) during immunization improved the protective capacity of our vaccine candidate composed of a trans-sialidase fragment (TSf), and a cage-like particle adjuvant (ISPA). **Objective:** To apply machine learning to identify potential CoPs by integrating biomarkers from both regulatory and effector immune responses for our vaccine candidate. **Methods:** BALB/c mice were vaccinated with protocols based on TSf-ISPA immunization with or without 5FU administration. Anti-TSf IgG levels, delayed-type hypersensitivity to TSf, and percentage of CD11b+Gr-1+, CD4+, CD8+ cells in peripheral blood were measured. Mice were challenged with 1700 *T. cruzi* parasites, and survival was recorded until day 35 post-infection (total n=46 mice).

A decision tree classification model was trained using immunization and survival data, using different variable sets. **Results:** Integrating both effector and regulatory response variables in the decision tree model yielded higher predictive accuracy for CoPs compared to models using individual variables. The integrated model achieved a weighted average precision of 88%, sensitivity of 87%, and f1-score of 88%. The macro average also showed a significant improvement over the model using individual variables ($p<0.05$). The area under the ROC curve increased from 0.36 to 0.80. **Discussion:** These findings suggest that machine learning can effectively help to identify potential CoPs for *T. cruzi* vaccines, with integrated variables enhancing predictive power. Future studies with larger sample sizes could further validate these models.

567. 251. **PERIPHERAL BLOOD B LYMPHOCYTE SUBPOPULATIONS AND ITS RELATION WITH THE PRODUCTION OF IGG-ANTI-SPIKE AFTER CORONAVAC VACCINATION**

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With the COVID-19 pandemic, there was a breakthrough in vaccine development and the need arose for better characterization of the establishment of protective immune responses for both the natural infection and vaccine. Most of the articles followed the context of detailing the contribution of T lymphocytes in infection and vaccination against SARS-CoV-2, while B cells did not receive the same attention. The combination of multiparametric cytometry with functional studies identified important differences between circulating cells in peripheral blood. The aim of this study was therefore to identify six subpopulations of B lymphocytes using multiparameter flow cytometry, and to assess their correlation with the production of IgG antibodies and neutralizing antibodies after vaccination. To this end, 40 individuals aged 18-75 years old were recruited and separated into three study groups: young people, healthy elderly people and elderly smokers. The group of smokers showed significant alterations in some subpopulations of B lymphocytes when compared to the groups of healthy young people and elderly people. On the other hand, no subpopulation showed a correlation with IgG levels, or with the frequency of neutralizing antibodies. In line with recent evidence showing in elderly people that the renewal of bone marrow-derived B cells, the ability of these B cells to differentiate and the secretion of antibodies are fairly well preserved, we suggest that it is the loss of the contribution of T cells due to immunosenescence that leads to deficient humoral immune responses in the elderly, including responses to vaccines, and that there is no subpopulation of B cells that can be a predictor of the humoral response.

568. 256. **IMPACT OF SEX HORMONES ON IMMUNE RESPONSE AND MYELOID-DERIVED SUPPRESSOR CELL MODULATION IN A TRANS-SIALIDASE VACCINE FOR CHAGAS DISEASE**

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Sex hormones can affect the immune response, therefore they can influence the efficacy of vaccines. However, most preclinical vaccine studies evaluate only females, as they generally showed a stronger humoral response. Furthermore, evidence suggests that male mice are more susceptible to *T. cruzi* infection. In this context, we aimed to evaluate whether a mucosal vaccine based on Trans-sialidase (TS) could control parasite growth after oral infection. BALB/c mice (both sexes) were intranasally immunized (3 doses, one every two weeks) with saline (V-group), TS (TS-group), c-di-AMP (A-group), and TS plus c-di-AMP (TS+A-group). Fifteen days following the completion of immunization, the levels of TS-specific antibodies (IgG2a and IgG1) were measured by ELISA. Additionally, the cellular response to TS was assessed through a delayed hypersensitivity test, injecting 5 µg of TS into the footpad. Subsequently, mice were challenged orally with 3000 Tc/dose of Tulahuen strain. Parasitemia and clinical conditions were monitored during

the acute phase. The splenic $CD11b^{+}/Ly6C^{+/low}/Ly6G^{+/-}$ MDSC response was also evaluated by flow cytometry. In both sexes, TS+A induced an increase of TS-specific IgG1 and IgG2a ($p<0.05$ vs. rest groups), although males had lower levels than females. Furthermore, in both sexes, TS+A triggered an enhanced TS-specific cellular response ($p<0.05$ vs. rest groups), which was maintained until 72h ($p<0.05$). Parasitemia was higher in males than in females in V, TS and A groups. However, in both sexes, TS+A strongly reduced the parasitemia and the clinical signs of infection ($p<0,05$). Additionally, MDSC cells were more reduced in the TS+A female mice ($p<0.05$ vs. rest groups), probably favoring the effector response. Overall, sexual dimorphism significantly influences vaccine immunogenicity, however, vaccine

569. 312. **NANOFORMULATION OF VACCINE COMPONENTS ENHANCES ANTIGEN-SPECIFIC B CELL POPULATIONS CRITICAL FOR ANTIBODY RESPONSES**

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We previously reported that a nanoformulation of OVA and CpG-ODN with a nanostructure (Coa-ASC16) elicited a more robust OVA-specific memory antibody response compared to the vaccine components in solution. Here, we examined the impact of different vaccine formulations on OVA+ B cells using OVA-AF-647, including germinal center (GC) B cells, memory B cells, and antibody-secreting cells (ASCs), at 20 and 167 days post-immunization using flow cytometry and ELISpot. Mice were subcutaneously immunized with a single dose of either OVA and CpG-ODN nanoformulated with Coa-ASC16 (OCC), a heated and cooled OVA and CpG-ODN solution (OCø), a heated and cooled OVA solution combined with CpG-ODN at RT (øO/C), or an OVA solution at RT combined with heated and cooled CpG-ODN (O/øC). The heating and cooling processes replicated nanoformulation preparation conditions. One-way ANOVA with post-hoc Tukey was used, and $p<0.05$ was considered to be significant. Short-term, OCC and øO/C promoted OVA^{+} GC B cells, memory B cells, and ASCs in the spleen. In the lymph nodes, OVA^{+} GC B cells were detected only in OCC-immunized mice, while the highest frequency of OVA^{+} memory B cell occurred in mice immunized with OCC, OCϕ, and ϕO/C. The highest proportions of OVA^{+} ASCs were in mice immunized with OCC, ϕO/C, and O/ϕC. ELISpot analysis of bone marrow cells revealed that OCC-immunized mice had the highest frequency of OVA-specific IgG ASCs. Long-term, OCC induced a higher proportion of OVA^{+} GC B cells, memory B cells, and ASCs in the spleen compared to other formulations. In the lymph nodes, only OVA^{+} memory B cells were observed, exclusively in OCC-immunized mice. ELISpot analysis showed a greater abundance of OVA-specific IgG ASCs in the spleen of OCC-immunized mice compared to other groups. This enhancement in B cell population generation in OCC-immunized animals supports the observed OVA-specific antibody response.

570. 320. **RATIONAL IDENTIFICATION OF A MULTIVALENT VACCINE CANDIDATE FROM CONSERVED IMMUNOGENIC PEPTIDES IN ENTRY AND EXIT PROTEINS OF THE ORTHOPOX GENUS**

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The *Orthopox* genus includes various emerging and re-emerging zoonotic viruses that threaten global health and affect a wide range of animals. Among these viruses, smallpox caused pandemics in the 20th century, the emerging Borealpox caused the first death in Alaska in 2024, and Mpox, classified by the WHO as a Public Health Emergency of International Concern in 2022, had its alert reclassified in 2024 due to the new Clade Ib variant, which has already caused over 14.000 cases in Africa. The lack of specific therapies for these viruses, combined with the limitations of attenuated virus vaccines, especially in immunocompromised populations, underscores the urgence for new approaches. This study aims

to develop a multi-epitope vaccine against all described virus from the *Orthopox* genus. The proteins responsible for viral entry and exit processes were extracted from the National Center for Biotechnology Information Virus database. A total of 160 sequences from all described virus from the *Orthopox* genus was obtained and they were analyzed to identify conserved epitopes using the Immune Epitope Database. After excluding transmembrane regions and N-glycosylation sites, the epitopes were concatenated to construct a chimeric multi-epitope protein, combined with the adjuvants β-defensin and PADRE. The resulting chimeric protein, containing eight conserved epitopes covering all viruses from the *Orthopox* genus, was evaluated for antigenicity, allergenicity, and structural stability. Furthermore, the potential multi-epitope vaccine showed good interaction with the TLR2 receptor, as well as satisfactory predictions of humoral and cellular immune responses for three doses of the vaccine candidate. The proposed vaccine may represent a new multivalent approach against these zoonotic viruses. The results indicate that the protein has potential for in vitro and in vivo studies, and, if its efficacy is confirmed, it could offer a robust solution for the prevention of diseases caused by these viruses.

571. 324. **CHARACTERIZATION OF A NEW MITOCHONDRIAL PROTEIN FROM LEISHMANIA AND ITS POTENTIAL USE AS A VACCINE**

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Human visceral leishmaniasis (HVL) is the second most lethal tropical parasitic disease. Notably, there are no prophylactic or therapeutic vaccines available for HVL. Thus, antigen discovery and the development of an efficacious vaccine are crucial. For this purpose, we performed immunoproteomics to identify immunodominant antigens in vaccinated mice extracts protected against *Leishmania amazonensis*. Among these, we selected a previously unstudied Kinetoplast-Associated Protein-like protein from *Leishmania infantum* (LinKAP) with repetitive sequences conserved across various trypanosomatids, including several *Leishmania* species. Differential centrifugation of *Leishmania* subcellular structures showed LinKAP enriched in fractions with other mitochondrial proteins. Endogenous labeling of the protein by CRISPR-Cas9 and immunofluorescence confirmed that LinKAP is a mitochondrial protein. We also generated LinKAP knockout cell lines for phenotypic analysis using CRISPR-Cas9. While the LinKAP gene function remains unknown, our results suggest it is not essential for promastigote-to-amastigote differentiation, infectivity, or amastigote multiplication in macrophages. We cloned and expressed a truncated LinKAP (rLinKAP), showing over 85% homology across *L. infantum*, *L. amazonensis*, *L. mexicana*, and *L. braziliensis*. Tested as a prophylactic vaccine for visceral leishmaniasis, adjuvanted with Poly ICLC, in mice and hamsters, immunized animals exhibited strong cellular and humoral responses and reduced tissue parasitism when challenged with *L. infantum*. We also tested rLinKAP as a therapeutic vaccine. Following therapeutic vaccination, antibody responses were enhanced, and cell responses became apparent. This treatment protocol inhibited splenic parasite growth in mice and reduced liver parasite burden by 80% in hamsters. Additionally, we generated a chimeric recombinant protein with rLinKAP associated with the *Leishmania* amastigote 2 (A2) protein, produced under Good Laboratory Practices (GLP). When tested as a therapeutic vaccine, this chimera demonstrated superior protective immunity compared to rLinKAP alone. In conclusion, the discovery and characterization of LinKAP, as well as vaccination with the rLinKAP/A2 chimeric protein, reveal a promising vaccine candidate for HVL.

572. 333. **COMBINATION OF BACULOVIRUS AND FLAGELLIN TO BE USED AS VACCINE PLATFORM WITH IMPROVED CAPACITIES**

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Baculoviruses (BVs) are insect viruses widely used as biosecured vectors for gene therapy, protein expression systems and for new vaccines platforms. BVs are commonly used as systemic adjuvants due to its naturally TLR-9 and cGAS-STING activation. To improve its mucosal performance as a vaccine our project is to combine BVs with the flagellin of *Salmonella typhimurium* flagella (FliC), a TLR-5 ligand with powerful mucosal activity. To test this hypothesis, we studied innate and adaptative immune response elicited by each stimulant alone or in combination. In macrophage murine cell line (J774.1) we showed no interference in the levels of IL-6, IL-1β and IFN α/β by the addition of FliC to increasing concentration of BVs measured by commercial kits. Besides, incubation of BV-GFP with FliC improved the uptake of BV-GFP in BMDCs and dendritic murine cell line (JAWS II) at low concentration of FliC such as 10Ng/ml compare with BV-GFP alone ($p<0,05$). For in vivo studies, four groups of female Balb/c mice were intranasally immunized at day 0 and 14 with different doses (1,5x10□ or $1,5x10^7$ pfu/dose) of either BV or coadministered with 2µg of FliC. Treatments turned out to be safe according to WHO approved mouse weight gain test and no rise of IL-6 in serum at 4hs post first vaccination. IgG titles were measured in serum 14 after the last immunization against the principal BV capside protein GP64. As we expected, the addition of flagellin increased significantly ($p<0,05$) the levels of antibody for the two baculovirus doses tested. These results show a better uptake capacity by the combination of the two immunogens that redound in a more powerful adaptative immune response *in vivo* unchanging the safety profile. The data obtained so far encourages us to generate a vaccine platform that combines both immunogens in a unique viral particle.

573. 359. **INSIGHTS INTO DIFFERENTIAL REPROGRAMMING AND TRAINED IMMUNITY THROUGH COMPARATIVE ANALYSIS OF INNATE IMMUNE RESPONSES TO DIVERSE COVID-19 VACCINE REGIMENS**

Ana Paula Flores Guirado#, Jonas Schmid#, Laura Power#, Sabrina Dhooge, Lucia Boffelli, Jeremias Dutto, Laura Cervi*, Eva V. Acosta Rodriguez*, Gabriel Morón*, Belkys A. Maletto*, Burkhard Becher*, Mariana Maccioni*, Nicolás Gonzalo Nuñez*.

The innate immune system, essential for defense against pathogens, shapes adaptive responses following vaccination. This study examined innate immune responses to 16 distinct COVID-19 vaccination regimens, including adenoviral, mRNA, and inactivated virus vaccines. Using spectral flow cytometry and algorithm-guided analysis, we compared the reprogramming of key innate immune cells, such as monocytes, NK cells, and dendritic cells. We identified eight distinct immune cell populations and analyzed activation markers at multiple time points post- vaccination. At T1 (4-12 weeks after the first dose), principal component analysis (PCA) showed clear segregation among vaccine types, with mRNA recipients exhibiting higher activation markers (CD39, CCR4, CCR7, $p<0.05$). By T28 (4-weeks after the second dose), distinct activation patterns emerged: 1)Viral Vector Group: Among seven combinations, significant segregation on PCA was observed for AZD/SputnikV (high% CD127 and CTLA4, $p<0.05$) and AZD/Ad5 (elevated% CD95 and CCR4, $p<0.05$), despite few differences in activation markers overall. 2)Boost mRNA Group: In four combinations where mRNA-1273 was administered as the second dose, four main clusters emerged, with the mRNA- 1273/mRNA-1273 group's myeloid compartment showing the highest levels of PD1, TIM3, and CCR7 ($p<0.05$). 3)Prime BBIBP Group: When BBIBP was used as the prime vaccine (four combinations), the BBIBP/AZD combination exhibited the highest levels of CD39 ($p<0.05$), while BBIBP/SputnikV showed the highest levels of TIGIT and CD27 ($p<0.05$) in the myeloid compartment. 4)Boost BBIBP Group: Among the three combinations, significant differences were noted only in the BBIBP/BBIBP group, which showed elevated TIM3 frequency ($p<0.05$). This analysis reveals differential innate immune signatures across various COVID-19 vaccine platforms and combinations, providing insights into their protective mechanisms. Understanding these responses is crucial for harnessing trained immunity to enhance defense against pathogens and cancers, complementing vaccine-induced adaptive immunity.

574. 362. **VARIANT-ADAPTED ARVAC COVID-19 VACCINE INDUCES A BROAD IMMUNE RESPONSE AGAINST EMERGING SARS-COV-2 VARIANTS AND SARS-COV**

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Flo[3], Julio Vega[3], Saposnik Lucas[1,2], Federico Paez Cordoba[1,2], Griselda Fascetto Cassero[1,2], Pasquevich Karina[1,2], Cassataro Juliana[1,2]

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COVID-19 continues to have a significant public health threat as the SARS-CoV-2 virus evolves, giving rise to variants that increasingly evade immunity from prior vaccinations and infections. Since the emergence of the Omicron lineage, several subvariants have emerged which have significantly reduced the efficacy of existing vaccines. In Argentina, a bivalent recombinant protein vaccine (ARVAC) based on the receptor binding domain (RBD) of the Gamma and Omicron BA.4/5 variants was authorized for use in 2023. In 2024, ARVAC was adapted to monovalent and bivalent formulations targeting the XBB.1.5 variant. Immunogenicity of adapted ARVAC vaccines was assessed in preclinical studies.
Immunogenicity data from mice primed with two doses of the adapted ARVAC vaccine demonstrated that both the monovalent XBB.1.5 and bivalent (Gamma/XBB.1.5) formulations successfully elicited significant neutralizing antibody responses against circulating variants, particularly XBB and JN.1. The bivalent formulation containing Gamma and XBB.1.5 exhibited a broader antibody response against the currently circulating variants as well as against more distant variants, such as Ancestral and Gamma. Following booster immunizations, the monovalent XBB.1.5 vaccine induced a significant 14.7-fold increase in neutralizing antibody titers against XBB.1.18 and a 9.8-fold rise against JN.1 compared to pre-boost levels. Notably, the bivalent vaccine elicited broad responses, effectively targeting both distant SARS-CoV-2 variants and demonstrating neutralizing activity against SARS-CoV itself. Furthermore, analyses of specific B cell responses revealed that only vaccines containing the XBB antigen effectively generated XBB-specific B cells, indicating the induction of a targeted immune response. In conclusion, the adapted XBB.1.5 ARVAC vaccine enhances the immune response against currently circulating variants of SARS-CoV-2. Moreover, the bivalent formulation containing gamma variant, exhibits broader immunity, effectively inducing responses not only against distant SARS-CoV-2 variants but also against other sarbecoviruses suggesting this vaccine platform could be useful to develop a pansarvecovirus vacine.

575. 369. DE NOVO ACTIVATION OF TRYPANOSOMA CRUZI SPECIFIC CD8 T CELLS WITH EFFECTOR FUNCTIONS VIA DC-TARGETED ANTIGEN DELIVERY

Lucía Biscari[1], Alfonso Herreros Cabello[2], Diana Karolina Santos[2], Sofía Chayeb Khouili[3], José Ignacio Herrero Lahuerta[2], Isidro García Gómez[2], Vanessa Nunez Gonzalez[3], Cintia Daniela Kaufman[1], Cecilia Farré[1,4], Nuria Gironés Pujol[2], David Sancho[3], Ana Rosa Pérez[1], Andrés Alloatti[1]

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$CD8^+$ T cells are critical in the protective immune response against *Trypanosoma cruzi* infection. Dendritic cells (DCs), particularly the conventional cDC1 subset, are pivotal in antigen presentation and the subsequent priming of naïve $CD8^+$ T cells. We hypothesize that enhancing the quality of $CD8^+$ T cell responses, especially in collaboration with $CD4^+$ T cells, can confer protection against *T. cruzi* infection. In a previous assay, we targeted the immunodominant *T. cruzi* epitope TsKb20 to cDC1 by covalently binding it to an anti-Clec9A antibody (Clec9A-TS). Clec9A is an endocytic receptor specific from cDC1 that promotes antigen presentation. Clec9A-TS induced *de novo* priming of TsKb20-specific $CD8^+$ T cells. In this work, we complemented our study by analyzing the effector phenotype of splenic $CD8^+$ T cells from mice treated with Clec9A-TS. Upon immunization, the $CD8^+$ T cells were capable of upregulate CD25, CD69, and CD137 and produced inflammatory cytokines such as IFN-γ, IL-2 or TNF-α following *in vitro* restimulation with

TsKb20. In addition, Clec9A- TS-immunized mice challenged with 2000 *T. cruzi* Y strain trypomastigotes exhibited a significant reduction in parasitemia at day 11 post-infection compared to the isotype control group. Statistical comparison between the Clec9A-TS groups and the isotype controls was performed using the non-parametric Mann-Whitney test. We also conducted an initial experiment to improve our assays by immunizing mice with a conjugate of a predicted MHC class II epitope linked to anti-Clec9A. Despite this effort, we did not observe any $CD4^+$ T cell-specific responses in the immunized mice. In conclusion, antigen targeting to cDC1s induces specific effector $CD8^+$ T cell responses sufficient to reduce parasitemia in *T. cruzi*-challenged mice. Further studies are required to evaluate the protective capacity of these conjugates and to explore additional epitopes that might elicit broader cellular responses.

576. 369. **DE NOVO ACTIVATION OF TRYPANOSOMA CRUZI SPECIFIC CD8 T CELLS WITH EFFECTOR FUNCTIONS VIA DC-TARGETED ANTIGEN DELIVERY**

Lucía Biscari[1], Alfonso Herreros Cabello[2], Diana Karolina Santos[2], Sofía Chayeb Khouili[3], José Ignacio Herrero Lahuerta[2], Isidro García Gómez[2], Vanessa Nunez Gonzalez[3], Cintia Daniela Kaufman[1], Cecilia Farré[1,4], Nuria Gironés Pujol[2], David Sancho[3], Ana Rosa Pérez[1], Andrés Alloatti[1]

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577. 380. **EXPLORING THE POTENTIAL OF *LENTILACTOBACILLUS KEFIRI* S-LAYER PROTEINS TO ENHANCE THE IMMUNE RESPONSE TO *CLOSTRIDIOIDES DIFFICILE* SURFACE ANTIGENS**

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The Gram-positive pathogen *Clostridioides difficile* is the leading cause of antibiotic-associated nosocomial diarrhea worldwide. Immunization with surface components is emerging as a potential strategy to control *C. difficile*-associated infections. The S-layer proteins form a two-dimensional lattice that completely covers the external surface of various pathogenic and non-pathogenic bacterial species. We have previously shown that S-layer proteins (SLPs) from different strains of *Lentilactobacillus kefiri* enhance the LPS-induced activation of murine macrophages. In this work, we studied the ability of SLPs derived from *L. kefiri* (SLP-Lk 83111) and a hypertoxigenic strain of *C. difficile* (SLP-Cd 43255) to stimulate murine macrophages (RAW264.7) *in vitro*, either individually or in combination. Cellular activation was assessed by quantifying IL-6 using a sandwich ELISA and quantitative PCR. While SLP-Cd 43255 did not induce significant activation at 30 µg/ml, SLP-Lk 83111 stimulated RAW264.7 cells when tested at 10 µg/ml ($P<0.05$). Notably, a combination of SLP-Cd 43255 (30 µg/ml) and SLP-Lk 83111 (10 µg/ml) induced a significant increase in both the expression and secretion of IL-6 compared to those observed after stimulation with the SLPs separately. Based on these results, six-weeks-old BALB/c male mice were immunized by intraperitoneal (IP) or subcutaneous (SC) routes following a regimen of 3 injections every 15 days. IP-1 and SC-1 groups received SLP-Cd 43255 (30 µg/mouse), while IP-2 and SC-2 groups were inoculated with SLP-Cd 43255 (30 µg/mouse) + SLP-Lk 83111 (10 µg/mouse). Anti-SLP-Cd 43255 antibodies in serum were determined by indirect ELISA. Samples obtained after the 3rd injection showed a significant increase ($P<0.001$) in the production of anti-SLP-Cd 43255 IgG in the SC-2 group compared to the SC-1 controls. These results suggest that SLP-Lk 83111 could act as an adjuvant on the humoral immune response against surface proteins of *C. difficile*, which contributes to the development of new vaccine systems against this pathogen.

578. 399. **CD11C-TARGETING WITH A SINGLE DOSE OF NANOBODY-FC CHIMERIC ANTIGENS ENHANCE ANTIBODY AND CYTOTOXIC T CELL RESPONSES**

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Targe&ng an&gens to an&gen-presen&ng cells is highly effec&ve for elici&ng strong immune responses. CD11c, predominantly expressed on dendri&c cells in mice, has emerged as a promising receptor for this purpose. Nanobodies (Nbs), due to their small size, are ideal for construc&ng recombinant an&genic chimeras. Using in-house selected an&-CD11c and control Nbs, we created chimeras with ovalbumin (OVA) as a model an&gen. Ini&ally, we cross-linked Nb-OVA fusion proteins with a bivalent an&-OVA Nb, with or without human IgG1 Fc (h1Fc), and assessed their immunogenicity in C57BL/6 mice without adjuvants. A single low dose of both an&-CD11c chimeras—unlike the control chimeras—induced a rapid an&-OVA IgG response, which was significantly higher with the h1Fc complex, sugges&ng a synergis&c effect likely due to interac&on with Fc receptors. Consequently, we expressed Nb-h1Fc-OVA chimeras, which replicated the enhanced immunogenicity seen with the an&-OVA complex, resul&ng in long-las&ng an&-OVA IgG &ters. To further understand the role of the Fc component, we generated mouse IgG1 and IgG2aLALA (FcγR silent) Fc chimeras. We observed no significant difference in an&-OVA IgG &ters among the three NbCD11c-Fc-OVA chimeras, indica&ng that interac&on with FcγRs may not be crucial for the CD11c-Fc synergy. However, the extended half-life due to neonatal receptor binding could be beneficial, and we are currently exploring this aspect. Finally, combining NbCD11c- Fc-OVA chimeras with CD40 agonis&c an&bodies resulted in potent cytotoxic T cell responses from a single dose. This promising outcome prompted us to explore the efficacy of this approach as a cancer vaccine using the melanoma B16-OVA model and as a prophylac&c influenza vaccine by replacing OVA with hemagglu&nin, aiming to present a compelling single-dose candidate for both cancer and viral vaccines.

579. 427. **SIMULTANEOUS DELIVERY OF RECOMBINANT BCG AND SARS-COV-2 PROTEINS ELICITS STRONG ANTIVIRAL HUMORAL AND CELLULAR IMMUNITY**

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Background: SARS-CoV-2, the causative agent of COVID-19, has led to millions of deaths worldwide. Since its emergence, multiple variants have raised global public health concerns. Although the WHO has declared the pandemic over, developing alternative vaccines remains crucial. Current vaccines are approved for administration only from six months of age, with no approved options for newborns. Our laboratory previously developed a vaccine against SARS-CoV-2 with recombination technology using the *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) platform, routinely administered to newborns. **Objectives:** To evaluate the safety and specific immune response induced by a recombinant BCG vaccine expressing the SARS-CoV-2 nucleoprotein (rBCG-N-SARS-CoV-2), co- administered with recombinant nucleoprotein and spike RBD from the Omicron variant, along with Alum adjuvant, in a murine model. **Methods:** BALB/c mice were immunized with rBCG-N-SARS-CoV-2, followed by a booster dose of recombinant proteins on day 28. 42 days after immunization (endpoint), lymphocytes were isolated from mice and co-cultured with SARS-CoV-2 nucleoprotein (N, Omicron RBD or PPD (as BCG control)-pulsed dendritic cells. T-cell activation markers were assessed by flow cytometry. Supernatants from co-cultures and serum samples were analyzed using ELISA to quantify cytokines, specific antibodies, and neutralizing antibodies against the D614G and Omicron B.1.1.529 variants. **Results:** The rBCG-N-SARS-CoV-2 vaccine, combined with recombinant proteins, was safe and effectively induced $CD4^+$ and $CD8^+$ T cell activation against N and RBD antigens. These T cells produced cytokines with antiviral properties. Additionally, the immunization strategy elicited high titers of specific antibodies against N and RBD and neutralizing antibodies against the SARS-CoV-2 variants, as demonstrated in cVNT assays. **Conclusions:** The co-administration of the rBCG-N-SARS-CoV-2 vaccine with recombinant SARS-CoV-2 proteins represents a promising strategy for controlling SARS-CoV-2 variants. Because of its safety profile and robust virus- specific immune response, this vaccine candidate could be especially valuable in protecting newborns against future SARS-CoV-2 variants.

580. 433. BOOSTER VACCINATION WITH A RECOMBINANT SUBUNIT VACCINE AGAINST SARS-COV-2 INCREASES ANTIGEN SPECIFIC MUCOSAL ANTIBODIES

Federico Páez Córdoba[1], Clara G. Fascetto Cassero[1], Lorena M. Coria[1], Gonzalo Pérez Marc[2], Ana Ceballos[3], Juan Manuel Rodriguez[4], Mónica E. Lombardo[5], Laura Bruno[1], Agostina Demaría[1], Lineia Prado[1], Celeste Pueblas Castro[1], Federico Montes de Oca[4], Julio C. Vega[4], Juan Fló[4], Pablo Bonvehí[5], Jorge Cassará[4], Juliana Cassataro[1], Karina A. Pasquevich[1]
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A Phase II/III clinical trial for a SARS-CoV-2 recombinant booster vaccine (ARVAC CG) was conducted in Argentina with three different RBD versions: $ARVAC_{Gamma}$, $ARVAC_{Omicron\ BA.4/5}$ and $ARVAC_{Bivalent}$. The clinical trial was randomized, double-blind, crossover, placebo-controlled and multicenter including adult volunteers previously vaccinated against SARS-CoV-2 with ≤3 booster doses. An exploratory endpoint of the clinical trial was the study of the systemic and mucosal antibody response induced 14 days after vaccination in a subset of 200 volunteers. In Phase II 30 volunteers received Placebo treatment and 50 vol-

unteers received $ARVAC_{Gamma}$. Whereas in Phase III, there were 30 volunteers for each brunch assayed (Placebo, $ARVAC_{Gamma}$, $ARVAC_{Omicron\ BA.4/5}$ and $ARVAC_{Bivalent}$). Systemic IgG response was assessed in plasma samples by commercial ELISA kit (COVIDAR) for the detection of anti-Spike antibodies. Mucosal IgA response was assessed in saliva samples by commercial ELISA kit (EUROINMUNE) for the detection of anti-S1 antibodies. Response of antigen-specific antibodies in plasma and saliva samples was analyzed considering parameters such as: previous SARS-CoV-2 infection status (anti-N IgG levels in plasma), primary vaccination platform, presence or absence of booster, and sex of the volunteers. In Phase II, there were significant increases in both IgG and IgA levels at day 14 in volunteers who were vaccinated with $ARVAC_{Gamma}$ independently of the parameters previously mentioned. Placebo volunteers did not show statistical difference. In Phase III, IgG levels increased significantly with all three ARVAC versions regardless of the parameters assessed. IgA levels increased significantly with all three ARVAC versions when all Phase III volunteers were not subdivided according to the parameters previously mentioned. These results showed that a booster dose with ARVAC increases anti-Spike IgG levels in plasma and anti-S1 IgA levels in saliva at 14 days vaccination.

581. 437. BCG AS AN ADJUVANT IN ANTITUMOR VACCINES

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Background: Bacillus Calmette-Guérin (BCG), derived from Mycobacterium bovis, is primarily used for tuberculosis vaccination and the treatment of superficial bladder cancer. VACCIMEL, a therapeutic anti-melanoma vaccine, consists of four irradiated allogeneic melanoma cell lines and is adjuvanted with BCG and recombinant human GM-CSF (rhGM-CSF). This vaccination has proved to induce a potent Th1 response, and patients have shown improvement with the treatment. **Objectives:** This study aimed to investigate the early interactions between BCG and immune cells involved in antigen presentation, to clarify the role of BCG as an adjuvant in enhancing the immune response elicited by VACCIMEL. **Methods:** In vitro experiments assessed the phagocytic uptake of BCG by monocytes using optical and electron microscopy. Monocytes were co-cultured with BCG and irradiated melanoma cells from VACCIMEL to determine if BCG enhances the phagocytosis of tumor cells. Antigen presentation to anti-gp100 T cell clones was evaluated through HLA-I expression and subsequent T cell activation by Flow Cytometry (BD FACS Canto II) and ELISA. The maturation of dendritic cells derived from circulating monocytes was also assessed following BCG exposure. **Results:** BCG was rapidly phagocytosed by monocytes within 3 hours, as observed by optical and transmission electron microscopy. Co-cultures demonstrated that BCG increased the phagocytosis of irradiated melanoma cells. Additionally, BCG-treated monocytes showed enhanced antigen presentation via HLA-I and increased activation of anti-gp100 T cells. BCG also strongly induced the maturation of monocyte-derived dendritic cells. **Conclusion:** By activating key immune cells, BCG may enhance the immune response against melanoma antigens in patients vaccinated with VACCIMEL. These findings shed light on the adjuvant role of BCG in therapeutic cancer vaccines.

582. 440. COMBINATION OF BACULOVIRUS AND FLAGELLIN TO BE USED AS VACCINE PLATFORM WITH IMPROVED CAPACITIES

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Baculoviruses (BVs) are insect viruses widely used as biosecured vectors for gene therapy, protein expression systems and for new vaccines platforms. BVs are commonly used as systemic adjuvants due to its naturally TLR-9 and cGAS-STING activation. To improve its mucosal performance as a vaccine our project is to combine BVs with the flagellin of *Salmonella typhimurium* flagella (FliC), a TLR-5 ligand with powerful mucosal activity. To test this hypothesis, we studied innate and adaptative immune response elicited by each stimulant alone or in combination. In macrophage murine cell line (J774.1) we showed no interference in the levels of IL-6, IL-1β and IFN α/β by the addition of FliC to increasing concentration of BVs measured by commercial kits. Besides, incubation of BV-GFP with FliC improved the uptake of BV-GFP in BMDCs and dendritic

murine cell line (JAWS II) at low concentration of FliC such as 10Ng/ml compare with BV-GFP alone ($p<0,05$). For in vivo studies, four groups of female Balb/c mice were intranasally immunized at day 0 and 14 with different doses (1,5x10 or 1,5x10^7 pfu/dose) of either BV or coadministered with 2µg of FliC. Treatments turned out to be safe according to WHO approved mouse weight gain test and no rise of IL-6 in serum at 4hs post first vaccination. IgG titles were measured in serum 14 after the last immunization against the principal BV capside protein GP64. As we expected, the addition of flagellin increased significantly ($p<0,05$) the levels of antibody for the two baculovirus doses tested. These results show a better uptake capacity by the combination of the two immunogens that redound in a more powerful adaptative immune response *in vivo* unchanging the safety profile. The data obtained so far encourages us to generate a vaccine platform that combines both immunogens in a unique viral particle.

583. 458. **THE RBCG-N-RSV VACCINE IS SAFE AND INDUCES PROTECTIVE IMMUNITY AGAINST BOVINE RESPIRATORY SYNCYTIAL VIRUS AND MYCOBACTERIUM BOVIS IN CATTLE IN A NATURAL TRANSMISSION SETTING**

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Background: Respiratory infections are the second leading cause of death in cattle worldwide, with bovine respiratory syncytial virus (bRSV) and *Mycobacterium bovis* (*M. bovis*) being two of the most important respiratory pathogens. We developed a vaccine using BCG expressing the RSV nucleoprotein (N) (rBCG-N-RSV), which is immunogenic against bRSV in calves under controlled conditions. **Objectives:** To determine whether the rBCG- N-RSV vaccine is safe and induces humoral immune protection in cattle against bRSV and
M. bovis in a natural transmission setting. **Methods:** A total of 120 *Polled Hereford* calves, aged 14 to 21 days, were immunized and distributed in the following groups, composed of 30 animals: rBCG-N-RSV, BCG-WT, Cattlemaster GOLD FP5 (CV) and Placebo. Animals were vaccinated with a first dose, and then with a booster dose two weeks later. We evaluated the vaccine's safety by monitoring local and systemic adverse effects. The humoral immune response was evaluated by indirect ELISA against N protein of bRSV and Purified Protein Derivative of *M. bovis* (PPD-b), through blood samples to measure IgG1 and IgG2, and nasal fluid samples to measure IgA, on times 0 (pre-immune), 14 and 28 after the first immunization. **Results:** The rBCG-N-RSV vaccine did not induce systemic or local adverse effects at first immunization or booster. Regarding the immune response, rBCG-N- RSV vaccine, compared to CV, induced a significant increase ($p<0.0001$) of IgG2 and IgA anti-N-bRSV at day 28 post-first immunization with respect to pre-immune time. Furthermore, rBCG-N-RSV induced a significant increase ($p<0.0001$) in IgG2 and IgA anti- PPD-bovine at day 28 post-first immunization compared to pre-immune time, similar to BCG-WT vaccine. **Conclusion:** The rBCG-N-RSV vaccine was safe in calves and induced a specific humoral immune response against bRSV and *M. bovis* in a natural transmission setting.

584. 460. **PD-1 BLOCKADE IMPROVES THE IMMUNE RESPONSE INDUCED BY HRSV AND SARS-COV-2 VACCINES**

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Background. The programmed cell death 1 (PD-1) pathway plays an important immunoregulatory role, suppressing $CD8^+$ T cell function. Besides, PD-1 is expressed by other immune cells, including follicular helper $CD4^+$ T cells and B cells. In respiratory viral infection, PD-1 is overexpressed and interferes with viral neutralization. Therefore, examining immune responses to vaccine antigens in the context of PD-1 blockade may provide insights into the role of PD-1 in the immune system and potentially reveal new opportunities to improve vaccination. Additionally, blocks PD-1 could be a therapeutic target in respiratory infections, such as SARS-CoV-2 and human respiratory syncytial virus (hRSV). **Objectives**. To evaluate the effects of PD-1 blockade on the immune response induced by vaccines against hRSV or

SARS-CoV-2. **Methods**. C57BL/6 mice were immunized intradermally by a 2-doses schedule (0-14 days) of rBCG-N-hRSV or CoronaVac (Sinovac), and then both groups were treated intraperitoneally with 100 μg of Nivolumab (therapeutic PD-1 blockade) twice a week throughout the protocol. The group immunized with rBCG-N-hRSV was challenged with hRSV 7 days after the booster. Euthanasia was carried out 14 days after the vaccine booster. Lung and blood were collected to determine hRSV loads by qPCR. The IgG secretion levels were also determined by assessing the total anti-N or anti-S of hRSV or SARS-CoV-2. **Results.** We found that PD-1 inhibition with nivolumab improved the response to the rBCG-N-hRSV vaccine, reducing viral load in hRSV-infected mice. Also, nivolumab treatment increased total IgG levels in vaccinated and challenged mice with hRSV. Meanwhile, mice immunized with the CoronaVac vaccine and treated with PD-1 inhibitor also increased the total IgG levels and enhanced neutralizing antibodies against SARS-CoV-2 induced by the vaccine. **Conclusion:** Our results suggest that PD-1 blockade can improve vaccine immune responses against respiratory viruses, such as hRSV and SARS-CoV-2.

585. 464. STUDY OF THE IMMUNOGENICITY OF RV2626C ANTIGEN FROM *MYCOBACTERIUM TUBERCULOSIS* IN A MURINE MODEL.

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Background: Previously we demonstrated that stimulation of cells from latently tuberculosis infected individuals (LTBI) with specific epitopes of Rv2626c antigen induced significant secretion of IFN-γ in sharp contrast to non-infected subjects. Furthermore, anti-Rv2626c IgG circulating antibodies were detected in plasma from LTBI. Considering these findings, we hypothesized that Rv2626c could be a potential tool to be used for the development of vaccines against latent tuberculosis infection. Then, we studied the immunogenicity of Rv2626c in mice. **Objective:** To evaluate the immunodominant regions of Rv2626c antigen in a murine model. **Methods:** BALB/c mice were immunized by the subcutaneous route with rRv2626c plus DNA plasmid expressing murine IL-12, three doses every 14 days. Ten days after the last immunization, splenocytes were obtained and *in vitro* stimulated with peptide pools of Rv2626c covering the entire sequence of the protein. Each pool was composed of six short peptides (11 to 15 amino acids). Afterwards, IFN-γ production and specific plasma IgG against Rv2626c peptides were measured by ELISA. **Results:** Regarding specific IFN-γ production, we identify two essential immunodominant regions located between peptides 19 to 24 and 31 to 36, which correspond to pools D and F ($p<0.0001$). Moreover, peptides 3 and 4 (DO_{450nm}= 0.95 + 0.02), 17 - 19, 25 (DO_{450nm} = 0.17+ 0.01) - 28 and 33 (DO_{450nm}= 0.18 + 0.02) to 34 induced specific circulating IgG. Furthermore, by studying *in silico* the linear sequence of Rv2626c peptides, we observed that the most immunogenic regions were located at peptides 7, 8, 21, 23, 33, 34, 35 and 36. According to *in vivo* observations, these peptides are included in pools D and F. **Conclusion:** We determined the immunodominant regions of Rv2626c in a BALB/c model. This information could be used in the future to develop new therapeutic or prophylactic anti-tuberculosis vaccines.

586. 475. COMPARISON OF TWO ANTI-TUMOR POLY ALLYLAMINE NANOPARTICLE-BASED VACCINE PLATFORMS

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Nanotechnology showed promising results for cancer immunotherapy. Poly(allylamine)- tripolyphosphate polymeric nanoparticles enhanced antigen presentation on phagocytic cells and activating T cells. Here, we focused on a vaccine PAH nanoparticle (Np) platform to control the tumor cell growth in a mouse model. C57BL/6 male mice were immunized with two doses of Np1-OVA, Np2-OVA or PBS at 14 day-interval through combined routes (IM+SC). On day 21, mice were subcutaneously injected with the tumor cell line B16-OVA. Tumors were macroscopically monitored and mice were euthanized when tumor

volume reached >1500 mm^3. Humoral (IgG) and cellular (T cells and macrophages) immune responses were evaluated by ELISA and flow cytometry, respectively. Mice from PBS, Np1-OVA and Np2-OVA groups developed tumors with a mean volume of 987,27mm^3, 66,05mm^3 and 442,16mm^3, respectively. We found higher values of serum OVA-specific IgG in Np1-OVA and Np2-OVA vaccinated mice than in control mice (0,153±0,106 0,856±0,250; 0,761±0,1640; for PBS, Np1-OVA and Np2-OVA, respectively ($p<0.05$), with increased levels of OVA-specific IgG1 in Np-OVA-vaccinated mice than in control mice (0,186±0,139; 1,583±0,803; 1,569±0,357 for PBS, Np1-OVA, Np2-OVA, respectively ($p<0.05$). The analysis of the cellular immune response showed a higher frequency of $CD4^+IFN^+$ cells in the draining lymph node of mice from the Np1-OVA group than PBS and Np2- OVA groups ($p<0.05$). The tumor-infiltrating T cell and macrophage counts showed significant differences between the PBS, Np1-OVA, Np2-OVA groups (T $CD4^+$ cells: 128,9±142,8; 10356,1±7216,7; 493,0±437,6; T $CD8^+$ cells: 167,2±209,4; 8164,6±8046,6; 828,7±775,9 macrophages: 161,2±174,1; 5428,2±2483,6; 1084,6±1006,2 respectively) ($p<0.05$). In conclusion, our findings showed that the nanoparticles are potent adjuvants that promoted an anti-tumor response in vaccinated mice, that improved, serum OVA-specific IgG, $CD4^+IFN^+$ T cells in tumor-draining lymph nodes, and macrophages and T cells infiltrating the tumor. Overall, the immune response induced by both PAH nanoparticles controlled the growth of the B16-induced melanoma in mice. Overall, Np1-OVA worked better than Np2-OVA to control tumor growth.

587. 484. **ASSESSMENT OF CELLULAR EFFECTOR IMMUNITY IN PEOPLE LIVING WITH HIV AFTER PNEUMOCOCCAL VACCINE**

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Background: The vaccine response for people living with HIV (PLWHIV) is generally smaller due to changes provided by the lower capacity for cellular activation. Vaccination with conjugate vaccines seeks to improve this response. **Objective:** This study aimed to evaluate the differentiation of effector T cells in PLWHIV after vaccination with 13-valent pneumococcal conjugate vaccine (PCV-13) followed by 23-valent polysaccharide vaccine (PPV-23). **Methods:** A prospective longitudinal cohort study was carried out in PLWHIV using HAART regularly (at least 4 weeks) at the HIV specialty outpatient clinic in Guarulhos (Sao Paulo- Brazil), patients were grouped according to the time of HIV infection. HIV in recent infection (RIHIV) with <1 year proven, chronic infection (CIHIV) with ≥ 1 year and non- HIV control group (NHIV) received vaccination with PCV-13 followed by PPV-23 after 8 weeks. Blood samples were performed to evaluate the immunophenotyping of total CD4 T lymphocytes (TCD4), effector memory TCD4 (TCD4me) using CCR7-CD45RA- markers at three moments: before vaccination with PCV-13, 8 weeks after vaccination with PCV-13 and 8 weeks after PPV-23 +PCV-13. **Results:** The mean total pre-vaccine TCD4 was lower in the two PLWHIV groups (RIHIV and CIHIV) compared with non-HIV ($p<0.0001$). This proportion was maintained 60 days after PCV-13 ($p=0.028$) and 60 days after PCV-13+PPV23 ($p=0.001$). In the assessment of TCD4me cells, pre-vaccination and 60 days after PCV-13 the CI-HIV group and NHIV were significantly different ($p=0.012$ and $p=0.001$ respectively). There was no difference between RIHIV and NHIV groups for TCD4me. **Conclusion:** PLWH despite having a lower reserve TCD4, presented more effector memory cells after combined vaccination with conjugate and polysaccharide vaccines. This difference was observed especially in patients with longer infection time. There was no difference in immune response between PLHIV regardless of the time of infection.

588. 494. **TRAINED IMMUNITY INDUCED BY AN INACTIVATED SARS-COV-2 VACCINE REDUCES HUMAN RESPIRATORY SYNCYTIAL VIRUS DISEASE**

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Background: The innate immune cells can develop long-term immune memory known as “trained immunity” (TI), described as a phenotype of enhanced inflammatory response induced by

stimuli such as the BCG vaccine and β-glucan. These stimuli induce epigenetic and metabolic changes in innate cells, promoting this profile and conferring protection against unrelated pathogens. Recent studies have suggested that current vaccines against SARS-CoV-2 may also induce a TI profile. Therefore, it is hypothesized that CoronaVac, an inactivated vaccine against SARS-CoV-2, could induce a TI profile capable of protecting against another respiratory virus, such as the human respiratory syncytial virus (hRSV). **Objectives:** To evaluate the non-specific protection induced by CoronaVac against hRSV in a murine model. **Methods:** BALB/c mice were immunized with CoronaVac and, 28 days post-immunization, challenged with purified hRSV. At 3 days post-infection, lung, blood, and spleen were obtained and analyzed by flow cytometry, ELISA, and RT-qPCR. **Results:** The results showed that the CoronaVac vaccine conferred a degree of protection in mice against hRSV infection, significantly reducing respiratory resistance on the second-day post-infection, along with decreased neutrophil infiltration and viral load. The landscape of the innate immune response to hRSV infection on CoronaVac-immunized mice was associated with increased cells such as alveolar macrophages, plasmacytoid, and conventional dendritic cells, as well as a differential expression profile of activation markers CD80, CD86, and MHC-II in innate cells. Furthermore, an increase in H3K4me3 and H3K27ac marks were found in the lungs and spleen, consistent with *in vitro* assays demonstrating the vaccine's ability to induce a differential pro-inflammatory response in trained cells upon restimulation with microbial ligands in a histone methylation-dependent manner. **Conclusion:** This study reports for the first time the capacity of an inactivated vaccine against SARS-CoV-2 to generate cross-protection against hRSV through a mechanism of TI.

589. 505. **MEASLES VACCINATION COVERAGE AND THE RESURGENCE OF CASES IN BRAZIL**

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Background: Measles is a highly contagious infectious disease that was once one of the leading causes of mortality. In 1992, Brazil introduced the measles, mumps, and rubella (MMR) vaccine. Today, vaccination occurs at 12 months (D1) and 15 months (D2). In 2019, Brazil lost its certification as a Measles-Free Country due to the return of the virus. Continuous immunization is essential for controlling the disease. **Objective:** To correlate measles vaccination coverage (VC) with the resurgence of cases in Brazil from 2017 to 2022.**Methods**: This is a descriptive quantitative ecological study that analyzed secondary data on vaccination coverage and case numbers, obtained from the National Notifiable Diseases Information System (SINAN) provided by the Ministry of Health. **Results**: Between 1992 and 2015, measles vaccination coverage remained above 95%, leading to the elimination of cases in 2016 and 2017, although coverage for D1 and D2 fell to less than 87% in 2017. In 2018, there was a significant increase, with 9,325 cases, 9,237 of which occurred in the North region, particularly in the state of Amazonas. In 2019 and 2020, numbers rose to 20,901 and 8,100 cases, respectively, with average coverage rates of 87.33% and 72.57%. During these years, Rio de Janeiro, São Paulo, and Pará were the most affected states. Starting in 2021, the number of cases dropped drastically to 676, with an average coverage rate of 64.07%, and in 2022, only 41 cases were reported, with no data on coverage, but cases were still concentrated in the North and Southeast regions. **Conclusion**: The influx of Venezuelan immigrants and the influence of anti-vaccine movements in 2017-2018 exacerbated the measles outbreak in Brazil. Additionally, COVID-19 and social isolation impacted vaccine adherence. Adherence to the vaccination schedule and continuous campaigns are essential to restore vaccination coverage and eradicate the disease.

590. 524. **IMMUNOGENICITY OF CONSERVED B EPITOPES OF ANTIGENS PRESENT IN THE INTESTINE AND OVARY OF THE TICK R. MICROPLUS (BM86, BM95 AND VITELLOGENIN)**

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Background. *Rhipicephalus microplus* is a tick

that affects cattle, causing significant economic losses to the livestock industry. Vaccines developed as a control method, use the intestinal protein Bm86 as an antigen, where it has been reported that allelic variations in the gene, as low as 3.4%, are sufficient to prevent a protective immune response. This highlights the need to study the presence of conserved B-cell epitopes of different antigens. Objectives. The aim of this study is to evaluate the immunogenicity of B-cell epitopes from antigens present in the intestine and ovary (Bm86, Bm95 and Vitellogenin) that are conserved in strains from different regions of México. Methods. Each peptide was evaluated individually by immunizing two Holstein cattle with 100 µg of peptide combined with *a* commercial adjuvant. Cattle were immunized at intervals of 21 days, receiving 4 to 5 immunizations in total. Sera from each immunization were obtained and evaluated by indirect ELISA to determine the generation of antibodies. Additionally, immunohistochemistry was performed to verify that the antibodies detect the target organ. Results. Three peptides from Bm86 and Bm95 generated antibodies, *showing an* increase after the second immunization and sustaining these antibodies levels though the fourth immunization. The three peptides also produced a positive immunoreaction in the intestinal tissue on the immunohistochemical test. Two Vitellogenin peptides generated antibodies from the second immunization and maintained at the third immunization. In both peptides, a positive response was observed during immunohistochemistry in ovarian tissues. Conclusion. The five selected peptides, with predicted B-cell epitopes of Bm86, Bm95 and Vitellogenin, are immunogenic and the antibodies produced can detect the target antigen. These peptides should be evaluated in efficacy trials to determine their potential as vaccine candidates against *R. microplus* infestations.

591. 535. **EFFECTS OF VACCINATION ON THE INTESTINAL MICROBIOTA AND THE SYSTEMIC IMMUNE RESPONSE**

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The immune response induced after vaccination can vary due to various factors, including intestinal microbiota. In the case of vaccination, it has been observed that differences in the composition and variety of the intestinal microbiota between individuals can influence the intensity of the immune response generated. **Aim:** To evaluate the impact of intestinal microbiota on the immune response induced by vaccination against respiratory viruses. **Methodology:** Mice vaccinated with CoronaVac or rBCG-N-RSV were subjected to different treatments: high-fiber diet, antibiotics, and butyrate supplementation. In rBCG-N-RSV-immunized animals, the immune response during RSV infection was also assessed. Specific IgG levels against SARS-CoV-2 and RSV were measured by ELISA. Gut morphology and secretion cytokines were evaluated by qPCR. The composition of intestinal microbiota was assessed in cecum content. **Results:** For RSV, antibiotic-induced intestinal dysbiosis in animals reduced protection from the rBCG-N-RSV vaccine against RSV infection, with viral loads comparable to those in non-immunized animals infected with RSV. Additionally, a high-fiber diet induced a lower *Firmicutes/Bacteroidetes* ratio following immunization, with higher levels associated with a dysbiosis profile. Furthermore, butyrate supplementation before vaccination, although it did not increase antibody levels, induced an anti-inflammatory profile characterized by higher IL-10 levels and lower IL-1β levels associated with the severity of the infection. On the other hand, in animals immunized with CoronaVac, lower levels of neutralizing antibodies were observed with antibiotic-induced intestinal dysbiosis. In the case of animals on a high-fiber diet, like vaccination with rBCG-N-RSV, a lower *Firmicutes/Bacteroidetes* ratio was observed. Finally, butyrate supplementation led to lower levels of IL-10 and IL-6 in the colon. **Conclusions:** The immune response generated by vaccines may depend on the intestinal microbiota profile. Various treatments that regulate intestinal microorganisms, such as a high-fiber diet and butyrate supplementation, could induce a better response to infection and improve the intestinal dysbiosis generated by vaccination.

592. 541. **EVALUATION OF HUMORAL AND CELLULAR RESPONSE TO SARS-COV-2 VACCINATION IN LIVER TRANSPLANT RECIPIENTS**

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Solid organ transplant recipients often show weaker vaccine responses and may not achieve adequate protection from SARS-CoV-2 vaccines. We characterized the immune status and evaluated the humoral and cellular response following the initial vaccination regimen (1st and 2nd doses) and the booster dose (3rd dose) of SARS-CoV-2 vaccines in liver transplant recipients (LTR) and healthy donors (HD). The humoral response was assessed by measuring anti-Spike antibodies and neutralizing antibodies, including those against SARS- CoV-2 variants such as Wuhan, Delta, and Omicron. The cellular response was analyzed using an ELISPOT assay for IFN-γ-producing cells in response to stimulation with Wuhan and Omicron SARS-CoV-2 peptides. The characterization of immune status included an analysis of the frequency of T and B cell subpopulations. Our results demonstrated that, following the initial vaccination regimen, LTR had a lower proportion of anti-Spike antibodies compared to HD (60% vs. 90%) and after the booster dose (73.5% vs. 100%). Anti-Spike antibody levels and neutralizing antibody titers were also significantly lower in LTR, including against viral variants. Multivariate analysis revealed that treatment with mycophenolate, advanced age, body mass index, and vaccination regimen influence the development of the humoral response in LTR. The cellular response in LTR was lower both after the initial vaccination regimen (for Wuhan, LTR: 32.5%, HD: 50%; for Omicron, LTR: 27.5%, HD: 62.5%) and after the third dose (for Wuhan, LTR: 39.6%, HD: 83.3%; for Omicron, LTR: 39.6%, HD: 83.3%). Regarding cellular phenotype, LTR showed significant differences compared to HD in the CD4/CD8 ratio and displayed a lower percentage of naive CD4+ and CD8+ cells along with a higher proportion of terminal effector cells and memory cells. In conclusion, significant differences were observed in vaccine-induced immune responses between LTR and HD. Understanding the dynamics of the immune response in immunocompromised populations is crucial for optimizing vaccination strategies.

593. 547. **TORQUE TENO VIRUS: A PREDICTIVE BIOMARKER FOR SARS-COV-2 VACCINE RESPONSE IN LIVER TRANSPLANT RECIPIENTS**

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Solid organ transplant recipients (SOTR) are highly susceptible to severe COVID-19 due to immunosuppression and comorbidities, and their vaccine responses are often reduced compared to the general population. Torque Teno virus (TTV), a component of the human viroma, has been previously identified as a marker of immune competence. Our objective was to develop an in-house qPCR assay to measure TTV viral load and evaluate its potential as a predictor of humoral response to SARS-CoV-2 vaccination. We designed TTV-specific primers, optimized a Sybr Green qPCR, and created a standard using a topo cloning vector containing TTV isolated from a liver transplant recipient, which was subsequently sequenced for validation. We determined TTV viral load in plasma samples from LTR and healthy donors (HD) after administering the initial vaccination regimen (first and second doses) and the booster dose (third dose) of the SARS-CoV-2 vaccine. The humoral response to SARS-CoV-2 included the measurement of anti-Spike antibodies and neutralizing antibodies. The prevalence of TTV was similar in both populations: 91.4% (70/77) in LTR and 92.9% (13/14) in HD. LTR had a significantly higher TTV viral load (median log10(copies/mL): 5.45) compared to HD (median log10(copies/mL): 4.10) (p=0.0001). After the initial vaccination regimen, LTR who failed to

develop a humoral response had a higher TTV viral load (median log10(copies/mL): 5.94) compared to those with a successful response (median log10(copies/mL): 4.9) (p<0.0001). This trend continued after the booster dose, with non- responders exhibiting an even higher TTV viral load (median log10(copies/mL): 6.26) compared to responders (median log10(copies/mL): 5.30) (p=0.005). Identifying a biomarker that reflects immune status and predicts vaccination response is crucial for optimizing vaccination strategies. In conclusion, a high TTV viral load predicts a poor humoral response to SARS-CoV-2 vaccination. This biomarker should continue to be investigated as a predictor of overall immune status.

594. 594. ENHANCED IMMUNOGENICITY AND PROTECTION BY MONTANIDE AND TLR3 AGONIST-COMBINED NUCLEOPROTEIN-BASED INFLUENZA VACCINES IN A MURINE MODEL

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Influenza is a respiratory disease of major concern playing a crucial role in the One Health vision. Despite significant efforts to control influenza in humans by vaccination, there is an increased risk of zoonotic infections due to close interactions between humans and poultry. Therefore, more effective influenza vaccines with a One Health focus should be developed to protect against emerging viruses. One promising approach to developing better vaccines is the use of conserved antigens combined with novel adjuvants and adjuvant combinations. The aim of this study was to evaluate the immunogenicity and protective efficacy of vaccines based on the influenza nucleoprotein (NP) combined with and oily adjuvant Montanide commonly used in veterinary vaccines, as well as with the addition of the Poly(I:C). Mice (n=5) were subcutaneously vaccinated with the formulations, and humoral and cellular responses were analyzed by ELISA, ELISPOT and flow cytometry. Furthermore, the vaccine efficacy was evaluated by challenging mice (n=6) against heterosubtypic H1N1 pdm 2009. Statistical analysis was performed using parametric and nonparametric analysis. The analysis of humoral responses indicated that the Montanide 70 plus Poly(I:C) formulation elicited significant and the highest IgG titers compared with NP alone. Further analysis of IgG subtypes showed that Montanide 70, and combined with Poly(I:C) significantly enhanced all subtype titers, mainly suggesting a Th1-skewed response. Moreover, mice vaccinated with the Montanide 70 and Poly(I:C) combined formulations exhibited significantly improved IFN-gamma secretion, showed by flow cytometry and ELISPOT. Challenge studies demonstrated that mice vaccinated with Montanide formulation as well combined with Poly(I:C) were fully protected against heterosubtypic H1N1 pdm 2009 infection. Our findings provide new insights into the use of novel adjuvants and adjuvant combinations for the development of improved universal influenza vaccines.

595. 622. NEUTRALIZING ANTIBODY RESPONSE AGAINST THE OMICRON VARIANT OF SARS-COV-2 IN THE VACCINATED CUBAN POPULATION

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Background: The rapid and widespread transmission of SARS-CoV-2 has led to the emergence of multiple variants of concern, most notably the Omicron variant, which continues to evolve and diversify into a range of sub-lineages, causing infection waves on a global scale, which has been attributed to immune escape. The objective of this work is to determine the neutralizing antibody response against the Omicron variant of SARS-CoV-2. **Methods**: Sample from eighty immunocompetent adult Cuban individuals, the titer of neutralizing antibodies against the Omicron sublineages was determined: BA1.2 (GISAID code: EPI_ISL_12691753), BA5.2 (GISAID code: EPI_ISL_17788608) y XBB1.6 (GISAID code: EPI_ISL_17789347); as well as against the D614G strain (GISAID code: EPI_ISL_7495115), through the neutralization method with live virus

standardized in the CICDC. The geometric mean of the neutralizing antibody titers against the Omicron sublineages was compared with the D614G strain, applying Friedman´s statistic and Dunn´s multiple comparison test. **Results**: The serum of the eighty individuals was able to neutralize D614G and the Omicron sublineages BA1.2 and BA5.2; while five samples did not neutralize the XBB1.6 sublineage. Significant differences were observed in the decrease in the geometric mean of the neutralizing antibody titer of the BA1.2 and BA5.2 sublineages with respect to D614G, decreasing 1,2 log and 1,45 log, respectively and highly significant for XBB1.6, greater than 6 log ($p<0.001$). When grouping the samples with respect to the neutralizing antibody titers ≤ 40 or ≥ 80, an increase in samples with neutralizing titers less than 40 was observed with the evolution of the Omicron sublineage: BA1.2 26.3% (21/80); BA5.2 23.8% (19/89) and XBB1.6 78.7% (59/75); while for D614G was only 11.2% (6/80). **Conclusion**: These results corroborate that the neutralizing antibodies generated by the Cuban Abdala and Soberana® vaccines maintain an adequate level of immunity against the Omicron variant of SARS-CoV-2.

596. 623. ANTIBODY ISOTYPE PROFILING BEFORE AND AFTER ADMINISTRATION OF ARVAC: A PROTEIN SUBUNIT VACCINE BOOSTER AGAINST SARS-COV-2

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the coronavirus disease 2019 (COVID-19) pandemic has continued to evolve rapidly. While different developed vaccines have shown clinical efficacy against ancestral SARS-CoV-2, waning immunity and immune escape variants led the need of boosters and variant adapted vaccines. Among these the recombinant subunit ARVAC vaccine has shown robust neutralizing antibody (NAb) responses against several SARS-CoV-2 variants. Although NAbs are considered an important correlate of protection against SARS-CoV-2 the role of other fragment crystallizable (Fc) functional antibodies in vaccine protection has received less attention. Recently, repeated SARS-CoV-2 mRNA vaccination has been linked to IgG4 skewing. In this work, antigen-specific IgG1, IgG2, IgG3, and IgG4 were evaluated in clinical samples of voluntaries that received one or two booster doses of ARVAC. Analysis of antibody isotypes previous to ARVAC booster showed, in agreement with recently published studies, that repeated mRNA SARS-CoV-2 vaccination was associated with high levels of RBD-specific IgG4, whereas these antibodies were low or undetectable in volunteers with one or none previous mRNA doses. The four Ag specific antibody isotypes were increased upon ARVAC booster, being in general, the IgG1 isotype the most predominant. Nevertheless, the proportion of IgG4 to IgG1 was similar before and after receiving the booster. These results suggest that one or two booster doses of ARVAC do not induce a class switch toward noninflammatory, RBD-specific IgG4 antibodies as it was shown after repeated SARS-CoV-2 mRNA vaccination.

597. 640. SERUM CONVERSION IN COVID-19 VACCINATED PATIENTS WITH CHRONIC KIDNEY DISEASE IS EQUIVALENT TO HEALTHY INDIVIDUALS

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Patients with chronic kidney disease undergoing hemodialysis have increased susceptibility

to COVID-19, progressing to severe disease and death. Consequently, the vaccination campaign against the pandemic in Brazil prioritized these. This study investigated anti-SARS-CoV-2 IgG antibodies in individuals undergoing hemodialysis and healthy individuals, comparing the serological response between the groups after administering the 3rd and 4th COVID-19 vaccine doses. This is a descriptive, prospective, and quantitative study conducted with volunteers from a hemodialysis center and healthy individuals recruited from the community in Minas Gerais, Brazil. A qualitative immunoassay (Bio-Manguinhos) was performed on plasma samples to detect anti-SARS-CoV-2 IgG in two post-vaccination periods: 160-300 days after the 3rd dose (D3) and between 45-100 days after the 4th (D4), regardless of the vaccination schedule received. The data were statistically processed using SPSS 20.0 software and compared using Pearson's chi-square test. The Research Ethics Committee approved this research under No. 5.274.253. In the first stage (D3), 29 hemodialysis patients and 18 healthy individuals participated, matched by sex, age, and vaccination time, of whom 22 (76%) of the dialysis patients and 14 (87%) of the healthy individuals tested positive in the anti-SARS-CoV-2 IgG ELISA. In the second analysis, after D4, 17 dialysis patients and 14 healthy individuals participated, of whom 7 (50%) dialysis patients and 10 (71%) healthy individuals tested positive. There was no difference ($p>0.05$) in the percentage of patients positive for anti-SARS-CoV-2 IgG difference between groups. The findings indicate that individuals on hemodialysis and healthy individuals have equivalent anti-SARS-CoV-2 IgG serological responses and suggests that they may have similar protection provided by vaccination. However, studies to the evaluation of neutralizing antibodies and the cellular response to the vaccination need to be conducted for a full understanding of the response of chronic kidney disease patients to COVID-19 vaccination.

598. 711. **CORRELATION BETWEEN HUMAN PAPILLOMAVIRUS VACCINATION COVERAGE AND CANCER RISK IN BRAZIL**

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Background: Human Papillomavirus (HPV) comprises over 200 virus types, infecting skin and mucosa in both genders. It causes various cancers, particularly cervical, but also vaginal, vulvar, anal, penile, oropharyngeal, and oral cancers. The National Cancer Institute (Inca) indicates that 80% of sexually active women will contract HPV at some point. Vaccination, available for free through Brazil's Unified Health System (SUS), is a safe and effective prevention method. **Objectives:** To analyze HPV vaccination coverage in Brazil from 2019 to 2022. **Methods:** This descriptive quantitative study analyzed secondary data from the National Immunization Program Information System (SI-PNI) and Inca. **Results:** In 2019, 87.08% of Brazilian girls aged 9 to 14 received a dose of the vaccine, but by 2022, coverage dropped to 75.81%. For boys, coverage decreased from 61.55% in 2019 to 52.16% in 2022, far below the 90% target for girls. INCA projects 17,010 new cases annually between 2023 and 2025, with a crude incidence rate of 15.38 per 100,000 women. The Northern Region of Brazil has the highest incidence (20.48/100,000). Several factors contribute to low vaccination adherence, including a lack of knowledge about the vaccine, fear of possible side effects, concerns about its efficacy, myths linking vaccination to early sexual activity, and misplaced trust in alternative preventive methods. Additionally, the influence of anti-vaccine movements, particularly on social media, plays a significant role. **Conclusion:** The World Health Organization estimates that HPV vaccination could prevent 70 million cases of cervical cancer in the 21st century. In Brazil, cervical cancer ranks sixth among the most frequent types. Among women, it is the third most common cancer. However, vaccination coverage in Brazil has declined, highlighting the need for effective public policies to increase adherence.

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599. 163. **EFFECT OF PANAX GINSENG FORMULATED WITH LIPOSOMES INJECTION ON NEUTROPHIL FUNCTION IN PERIPARTURIENT DAIRY COWS**

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The objective of this work was to evaluate the functionality of peripheral blood neutrophils after treatment with *Panax ginseng* extract (PGe) formulated with liposomes (LPS) during the transition period in dairy cows. Clinically healthy cows were assigned into four groups (n=10/group) and were treated 14 days (d) before expected calving with four formulations: PG+LIP group received subcutaneous injection of PGe (200 mg/ml in 10 ml of LPS), PG group received 200 mg/ml of PGe in 10 ml of saline solution (SS), LIP group received 10 ml of a liposomal formulation and control group received 10 ml of SS. Blood samples were taken at -14 (pretreatment), 1, 3 and 7 d relative to calving and neutrophils were purified. Flow cytometry was performed to analyse CH138, CD62L, CD44 and CD11b expression. Median fluorescence intensity (MFI) was used to estimate CD62L, CD44 and CD11b expression per positive cell. A significant effect of treatment was observed in CD62L expression over time (p=0.001). At 1 and 3 d postpartum (pp), CD62L expression increased in PG+LIP, PG and LIP groups compared with control group (p<0.001, p=0.002; respectively). In control group, a significant decrease in CD62L expression was observed at d 3 pp compared to d -14 and 7 pp (p=0.01). No significant effect of treatment was observed in CD44 and CD11b expression over time (p=0.078, p=0.778; respectively); however, at d 3 pp the expression of both receptors increased in PG+LIP, PG and LIP groups compared with control (p=0.001CD44 and p=0.05CD11b). At 7 d pp, CD62L MFI was higher in PG+LIP and PG groups compared with control (p<0.05). At 3 and 7 d pp, CD44 MFI was higher in PG group compared with control (p<0.05, p=0.05, respectively). In conclusion, treatment before calving with PG+LIP, PG, and LIP increased the functionality of neutrophils, mainly at d 3 pp.

600. 175. IMMUNOMODULATORY EFFECTS IN DAIRY COWS FOLLOWING INTRAMAMMARY INOCULATION OF MINTHOSTACHYS VERTICILLATA ESSENTIAL OIL AT DRYING OFF

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Background: We previously reported that *Minthostachys verticillata* essential oil (EO) effectively prevented intramammary infections in a murine mastitis model by stimulating the innate immune response. **Objective:** This study aimed to evaluate the immunomodulatory effects of EO via intramammary inoculation in dairy cows at drying off. **Methods:** Ten pregnant Holstein cows, free from mastitis, were selected from Establecimiento La Negrita, Zendero SRL. Sixteen quarters were inoculated with varying doses of EO (0.25 g, 0.50 g, and 1.00 g) in four quarters each. Additionally, four quarters received cefapirin (0.30 g), and four quarters were left untreated. Milk samples were collected before treatment and four days post-calving to measure fat content, total dry extract, relative density, acidity, ash content, pH, microbial count, and somatic cell count (SCC). The expression of TNF-α, IL-6, and IL-10 was quantified by qPCR, while malondialdehyde (MDA) levels were also assessed. Blood samples were taken to measure serum IL-6 and IL-4 levels using ELISA. **Results:** The EO treatments (0.25, 0.50, or 1.00 g) or cefapirin did not alter the physicochemical properties of the milk. Notably, EO at 1.00 g significantly reduced the microbial load ($p < 0.05$), comparable to cefapirin ($p < 0.05$). SCC increased after EO treatment at 0.25 g and 1.00 g ($p < 0.001$, $p < 0.01$). Although the expression levels of TNF-α, IL-6, and IL-10 decreased after EO (1.00 g) treatment, the differences were not statistically significant. MDA levels increased significantly with cefapirin ($p < 0.001$), whereas EO

at all doses did not affect MDA levels. Serum IL-6 and IL-4 levels remained unchanged post-treatments. **Conclusion:** Intramammary inoculation of EO in dairy cows at drying off stimulated the immune response by increasing SCC, reducing microbial load, and decreasing pro-inflammatory cytokine levels without affecting milk quality. This natural product could serve as a preventive treatment against bovine mastitis.

601. 196. **EFFECT OF A PROBIOTIC FERMENTED PRODUCT ON THE INTESTINAL ECOSYSTEM: IMMUNOLOGICAL AND METAGENOMIC STUDIES**

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Background: The beneficial properties of ***Lacticaseibacillus*** (***L.***) ***rhamnosus*** RC007 in healthy animals and in different models of gut inflammation were previously described. **Objectives:** to evaluate the effects of whey fermented by ***L. rhamnosus*** RC007 (FW) on the gut immune system, gut microbiota and the production of short-chain fatty acids (SCFA) in a mouse model. **Methods:** BALB/c mice were divided into three groups: control group received orally 0.1 ml of phosphate buffered saline (PBS); FW group received orally 0.1 ml of FW; and whey (W) group received orally 0.1 ml of whey without the probiotic bacterium. After 10 days, mice were sacrificed. Small intestines were collected for determination of IL-10; IL-6, TNF-α, goblet cells and intraepithelial lymphocyte. Cecum content was obtained for gut microbiota and SCFAs analysis. **Results:** all the cytokines assayed increased in FW group compared to control and W groups ($P < 0.05$). The ratio between anti and pro-inflammatory cytokines (IL-10/TNF-α) increased in the FW group. Increase in goblet cells, intraepithelial lymphocytes and butyric acid were observed in FW group ($P < 0.05$). Metagenomic analysis shown that FW increased microbiota diversity. The phyla Firmicutes was the most abundant in the three groups, following by Actinobacteriota abundance in FW group. Members of ***Lachnospiraceae*** and the ***Eggerthellaceae*** family were increased in FW group, microorganisms related with butyric acid production and anti-inflammatory properties, respectively. **Conclusions**: FW was able to stimulate and to modulate mouse immune system, reinforcing the intestinal barrier, since goblet cells and intraepithelial lymphocyte are the first line of host defense. Administration of FW modulated gut microbiota towards microbial populations with beneficial effects on gut ecosystem. Whey fermented by this probiotic bacterium is an interesting alternative for development of a new food additive for pig production.

602. 203. **BACILLUS-DERIVED LIPOPEPTIDE EXTRACTS MODULATE BALB/C MICE GUT IMMUNE RESPONSE IN ABSENCE OF PATHOGENIC CHALLENGE WITHOUT ALTERING MUCOSAL STRUCTURE OR CAUSING ORGAN OR BONE MARROW TOXICITY SIGNS**

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Background: *Bacillus*-derived lipopeptides, especially surfactin (SF), have shown anti-inflammatory, antimicrobial and adjuvant activity, among others. Studies have reported immunomodulating activity of SF at concentrations ranging from 20 to 80 mg/ml. **Objectives:** To test SF-containing lipopeptide extracts (LPE) from *Bacillus* spp. MFF 2.2 for immunomodulating, cytotoxic and genotoxic effects, to evaluate their potential application in veterinary medicine. BALB/c mice groups (n=6) were administered a daily oral dose of: 1) Saline solution; 2) 60 mg/ml SF standard; 3) 40 mg/ml SF-LPE; 4) 60 mg/ml SF-LPE. After 10 days, animals were sacrificed. Liver, kidney and small intestine samples were taken for histopathological analysis. Genotoxicity and cytotoxicity were evaluated by the micronuclei assay. Goblet cell and intraepithelial lymphocytes (IELs) were counted in small intestine. IFN-γ, TNF-α and IL-10 in intestinal fluid (IF) were quantified by ELISA. **Results:** No significant differences were observed in body weight gain of mice receiving the LPE compared to the control group. No toxicity signs were detected in organs or bone marrow. No significant differences in IELs counts were observed between treatments and controls. However, SF and both LPE concentrations increased goblet cell counts significantly ($P<0.05$)

compared to the control. A decrease in IFN-γ levels (P<0.05) was observed in IF of animals treated LPE compared to controls. No significant differences were observed in TNF-α or IL-10 concentrations of treated animals and controls. However, the IL-10/IFN-gamma ratio demonstrated an increase of the anti-inflammatory/pro-inflammatory cytokine ratio (P<0.05). **Conclusion:** *Bacillus*-derived LPEs could be safe products with potential applications in veterinary medicine. Their antimicrobial effect against ETEC and *Salmonella* Typhimurium demonstrated in previous studies suggest their potential use for managing diarrhea, inflammation and related conditions. Further studies are warranted to explore the effect of LPEs in an inflammatory or injury context, their long-term effects and therapeutic potential of these compounds.

603. 219. **IDENTIFYING ANTIGEN CANDIDATES FOR A VACCINE AGAINST INFECTIOUS CORYZA USING A REVERSE VACCINOLOGY APPROACH**

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Avibacterium paragallinarum is the causative agent of infectious coryza, an acute respiratory disease that affects chickens. This Gram-negative bacterium is prevalent in poultry production systems worldwide, leading to significant economic losses. Although vaccination is the primary preventive measure, commercially available vaccines often provide incomplete protection, especially against strains that are not included in the formulation. This study aims to utilize a Reverse Vaccinology approach to identify antigen candidates from both local and publicly available *Av. paragallinarum* strains, as a preliminary step towards developing a more effective protein-based subunit vaccine. Genomes from *Av. paragallinarum* strains isolated from poultry farms in various countries were retrieved from the NCBI database. Additionally, DNA samples from 25 *Av. paragallinarum* strains from Argentina, Bolivia, and Peru underwent high-throughput sequencing, followed by genome assembly and annotation. An *in silico* workflow was developed to predict and prioritize proteins with high potential for ease of expression and protective efficacy. This pipeline assessed various protein attributes, including sequence conservation across strains (using MAFFT and CD-Hit), antigenicity (with VaxiJen v.3), essentiality (via BLASTp comparison with the DEG database), lack of homology to the host (by BLASTp comparison with Gallus gallus genomes), and outer membrane or extracellular localization (using PSORTb), among other features. Fourteen proteins were identified as potential antigen candidates. Four of the promising candidates, meeting all the criteria, were the outer membrane protein assembly factor BamA, OmpH family outer membrane protein, LPS assembly protein LptD, peptidoglycan-associated lipoprotein Pal, and TonB-dependent hemoglobin/transferrin/lactoferrin family receptor. While in vivo testing is still required, this study lays the groundwork for developing a novel subunit vaccine against *Av. paragallinarum*.

604. 224. **LONGITUDINAL STUDY OF THE HUMORAL IMMUNE RESPONSE TO CLOSTRIDIAL VACCINATION IN CATTLE**

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Clostridia, the oldest known disease-causing agent affecting livestock and other species, encompass more than 200 species. Among them, 15 produce toxins that are responsible for severe disease. Despite the importance of clostridial diseases in cattle, limited research has explored the humoral and cellular responses induced by these bacteria due, in part, to their complex and

diverse nature. Vaccination remains the most effective strategy for preventing clostridial diseases and minimizing economic losses. Commercial multivalent clostridial vaccines, contain inactivated toxins or cellular components from various *Clostridia* species. Data on the duration of immune responses is scarce. In this context, our group has previously shown the induction of specific responses that declined to near basal levels by 3 months. Here, we present results from a longitudinal study in cattle for the evaluation of the immune response induced by a 9-valent experimental policlostridial vaccine, that includes 3 recombinant antigens. Moreover, we compared 2 schedules, one including the recommended annual booster and other receiving an additional booster at 6 months. Specific humoral responses were assessed at 8 time-points over 15 months using custom-designed ELISAs. Vaccinated animals showed a significant increase in specific IgG titres against each antigen at 14 days post-vaccination, which declined to near basal levels 6 months later. Booster doses at 6 and/or 12 months further increased IgG titres, but these waned 3 months after administration. Both groups reach similar specific IgG titres after the annual booster, suggesting that the additional 6-month booster did not improve the response, though it contributes to maintaining specific titres along the year. We are currently setting up flow cytometry, fluorospot, and RT-qPCR assays aiming to analyse cellular recall responses in vaccinated animals. Overall, our results highlight specific humoral immune responses induced by clostridial vaccination and provide insights for future vaccine design.

605. 226. **ACTIVATION OF GAMMA-DELTA T CELLS AFTER IN VITRO EXPOSURE TO TWO STAPHYLOCOCCUS AUREUS STRAINS WITH DIFFERENT ADAPTATION GENOTYPES**

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The aim of this study was to evaluate the activation of bovine WC1+ gamma-delta T lymphocytes (WC1+ lymphocytes) and the cytokine production after *in vitro* exposure to two *S. aureus* strains with different adaptation genotypes (low or high) to the bovine mammary gland: one persistent (P, strain 5011) and one non-persistent (NP, strain 806). WC1+ lymphocytes were purified from bovine peripheral blood mononuclear cells (PBMCs) with immunomagnetic separation. Total PBMCs, purified WC1+ lymphocytes, and the remaining PBMCs after WC1+ lymphocytes purification (WC1- cells) were cultured without stimulation (basal cells) or with heat-killed *S. aureus* strains P and NP. After 48 hours, the expression of CD80, MHCII, and CD62L on WC1+ lymphocytes were evaluated by flow cytometry, and IFN-gamma and IL-17 levels were measured in culture supernatants by ELISA Kits. Results were analysed using a generalized linear model. A significant increase in the percentage of WC1+ CD80+ and WC1+ CD62L+ lymphocytes, along with the mean fluorescence intensity (MFI), was observed after culturing total PBMCs with *S. aureus* strain 806 compared to basal cells ($p<0.05$). No differences were observed in the percentage or MFI of WC1+ MHCII+ lymphocytes under the same conditions ($p>0.05$). A significant increase was observed only in the percentage of WC1+ CD80+ lymphocytes stimulated with *S. aureus* strain 5011 compared to basal cells ($p<0.05$). No differences were detected in the percentage or MFI of WC1+ CD62L+ and WC1+ MHCII+ lymphocytes after culturing purified WC1+ lymphocytes with both strains ($p>0.05$). Levels of IFN-gamma and IL-17 were significantly higher in total PBMCs cultures compared to purified WC1+ lymphocyte and WC1- cells cultures for both strains ($p<0.05$). In conclusion, WC1+ lymphocytes are indirectly activated by *S. aureus in vitro* and contribute to the release of IFN-gamma and IL-17. This response varies with the genotypic and phenotypic characteristics of the *S. aureus* strains.

606. 227. **INFLUENCE OF STAPHYLOCOCUS AUREUS STRAIN WITH DIFFERENT ADAPTATION GENOTYPES ON CYTOKINE PRODUCTION BY MAC-T CELLS**

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Staphylococcus aureus is the most frequently isolated pathogen from cases of bovine mastitis and can be classified as non-persistent (NP) or persistent (P) according to their ability to adapt to bovine mammary gland. The aim of this study was to evaluate the ability of different *S. aureus* strains (NP and P) to induce cytokine production in MAC-T cells at different post-infection (pi) times. We evaluated the levels of IL-1beta, IL-6 and TNF-alpha production in MAC-T cells infected with *S. aureus* strains NP (3, 17, 48, 179, 806) or P (37, 316, 1595, 5011, 5128). IL-1beta and IL-6 levels were measured by ELISA, while intracellular TNF-alpha production was assessed by flow cytometry. IL-1beta and IL-6 levels, relative to basal cultures and in association to *S. aureus* origin, were analysed using a gamma-linked GLM. All strains were able to induce IL-6 and IL-1beta production (except strain 1595) by MAC-T cells after 2 h pi. Furthermore, an association between cytokine levels and strain origin was observed, where NP strains inducing higher cytokine levels than P strains ($p<0.05$). The ability of *S. aureus* strains to induce TNF-alpha production in MAC-T cells, relative to uninfected cells, was analysed using a T-test. Comparisons between the percentages of TNF-alpha positive cells infected with different strains were made by normalizing to basal production through a linear GML. Three NP strains (3, 17 and 48) and one P strain (1595) were able to induce intracellular TNF-alpha production in MAC-T cells after 24 h pi, where strain 17 inducing the highest TNF-alpha production compared to the other strains ($p<0.05$). In conclusion, although each strain exhibits a unique behaviour upon infection, the origin of the strains influences the activation of MAC-T cells. Overall, an increased level of pro- inflammatory cytokine production by MAC-T cells was observed in response to the NP strains.

607. 228. DISPERSED ASCORBYL PALMITATE (ASC16) AS AN ADDITIVE IN ADJUVANT FORMULATIONS

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Different aspects determine the choice of an adjuvant to generate optimal antibody production while ensuring the safety of the producing animal. In this context, ASC16 that can form safe hydrogels or act as a toxin inhibitor depending on whether it is present at high or low concentrations, respectively. For these reasons, it was proposed to evaluate the effect of dispersed ASC16 (low concentration) as an additive adjuvant in the production of experimental antivenom. BALB/c mice were immunised with Freund's adjuvant in the presence or absence of additive (AFMo and AF) and hydrogel with or without additive (Pa40Mo and Pa40). Afterwards, ELISA, avidity and immunoblotting tests were performed to determine the titre, binding strength and specificity of the antibodies. Finally, we determine the effective dose 50 (ED50) that neutralises PLA2 activity of Crotalus durissus terrificus venom. We used the t-tests for two independent samples to verify if there were significant differences. Our results show that AFMo induced the highest titres (4.07), followed by AF and Pa40Mo (both 3.75), although without significant differences ($p>0.05$), and finally Pa40 (3.27). Notably, the additive ASC16 also improved antibody avidity, with AFMo (4.35 M KSCN) standing out with significant differences compared to the rest ($p\leq0.05$), followed by AF and Pa40Mo (3.21 and 3.17 M KSCN) with no significant differences ($p>0.05$), and finally Pa40 (1.61 M KSCN). In addition, the antibodies recognised the main antigenic components of the venom. The sera produced by formulations with additive presented a better ED50 for PLA2 activity, highlighting Pa40Mo (7.04 µL), followed by AFMo (9.02 µL) with significant differences ($p\leq0.05$) compared to Pa40 (12.20 µL) and AF (12.23 µL). This study shows that formulations with additive ASC16 as an enhancer induce higher antibody titres, improve avidity, have a greater specificity and neutralising effect on PLA2 activity than formulations without the additive.

608. 233. NEW MYCOBACTERIUM BOVIS ANTIGENS FOR USE IN BOVINE TUBERCULOSIS CONTROL STRATEGIES

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Bovine tuberculosis (BTB) poses a threat to livestock production at all levels of production. Mycobacterium bovis is the main causative agent of

TB and is also a pathogen capable of infecting wildlife and humans. The official method for diagnosing TB is the intradermal tuberculin reaction (IDR). The reading of the reaction to the inoculation is done at 72hs. In 2015, the World Animal Health Organization (OIE) certified BOVIGAM, a test to detect cell-mediated immune responses to M. bovis infection. This test stands out for its ability to detect infected cattle that escape the intradermal test and those in the early stages of infection. The proteins ESAT-6, CFP-10 and Rv3615c have been extensively tested for the diagnosis of TB and human tuberculosis due to their potent T-cell response in IFN-gamma release trials. In this paper we express some members of the ESAT-6 family, a set of proteins secreted by M. bovis that has been shown to be immunogenic in animal models. The Esx M, L, E, F, J, K, G, C, H, I proteins were expressed in recombinant baculovirus-infected insect cells and the EsxO and EsxU-expressed recombinants were found. The proteins obtained from the polyhedra were analyzed in SDS-PAGE and the recombinant proteins were identified by western blot. The appearance of a band of expected molecular weight for the fusion of proteins to POLH in SDS-PAGE gels indicated the production of recombinant protein. Its identity was confirmed by Western blot using an anti-POLH serum. We will stimulate blood from cattle with TBB and healthy animals with each of the recombinant proteins to select those that are capable of inducing in vitro production of IFN-gamma.

609. 322. **SOCIO-ECONOMIC ASPECTS OF THE SPREAD OF ENTEROBIOSIS AND MEASURES TO PREVENT IT**

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The most common helminthiasis are: enterobiosis (57%), ascariasis (11.2%), and of the protozoa, giardiasis is mainly (28.9%). Intensity analisis of the enterobiosis spread in the territory of the republic showed that a high incidence rate (more than 1,000 per 100,000 population) is observed in one area, from 700 to 1000 is observed in one region, from 500 to 700 in three regions and the capital. The incidence of enterobiosis in 86,6% of all cases is formed at the expense of children under 14 years. Moreover, the incidence of children attending children's institutions is significantly higher (51.9±0.07). The average rate per 1,000 surveyed persons was 49.7, the minimum was 34.6 (2019), and the maximum was 61.7 (2017). The risk group for enterobiosis is children under 14 years old, the proportion averaged 41.02% over 6 years. Moreover, the incidence of enterobiosis in men was significantly higher (51.8%±0.05%) compared with women (48.2%±0.05%). Thus, in 2014, there was an increased invasiveness of the population of all categories. By 2016, there is a downward trend in the disease. However, by 2019, there is an increase in the detection of enterobiosis. In the Kyrgyz Republic, the average long- term intensive incidence of enterobiosis per 100,000 population for the period from 1960 to 2007 was 687.0± 3.2. The minimum figure was recorded in 2004 and amounted to 361.2, and the maximum figure was reached in 1989 and amounted to 1200.5. For the period from 2014 to 2019 in the city Bishkek there is an increase in the incidence of parasitosis. Enterobiosis (57.0%) and ascariasis are among the most common helminthioses in the city, and giardiasis is among the protozoa. The incidence of enterobiosis in Bishkek most strongly affects children under 14 years of age (41.02%). The incidence of enterobiosis in men was significantly higher (51.8% ± 0.05%).

610. 355. **EFFECT OF AN EXPERIMENTAL VACCINE AGAINST FASCIOLA HEPATICA FORMULATED WITH TWO RECOMBINANT KUNITZ-TYPE PROTEINS ON THE REDUCTION OF EGGS AND ADULTS IN VACCINATED SHEEP**

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Fasciolosis is a cosmopolitan parasitic disease

that significantly affects both animal and human health. Sheep are considered highly susceptible, and the development of effective vaccines has been proposed for their control. Kunitzs proteins are highly expressed by the parasite and are encoded by a family of seven genes. It has been demonstrated that the expression level of these proteins varies according to the parasite's life cycle stage. ***Fasciola hepatica*** Kunitz type 4 (FhKT4) is abundantly expressed early during infection, while ***Fasciola hepatica*** Kunitz type 1.1 (FhKT1.1) maintains high expression levels throughout the life cycle of ***F. hepatica***. These proteins were expressed recombinantly in the periplasm of ***E. coli*** and their usefulness in the early serological diagnosis of fasciolosis in sheep was demonstrated. The objective of this study was to evaluate a vaccine against ***F. hepatica*** formulated with FhKT1.1 and FhKT4 emulsified in aluminum hydroxide gel in experimentally infected sheep. Fifteen lambs were randomly assigned to four experimental groups: infected (I), vaccinated (V), adjuvant (A), and healthy control (C). Serial fecal samples were collected to determine egg counts up to 16 weeks post infection. Adult worms were collected from the liver and gallbladder. A generalized linear mixed model was fitted for statistical analysis of the data. The results indicated a significant reduction in egg count by 73.5% ($p<0.001$) and a decrease in adult count by 54.6% ($p<0.05$) in V ***vs*** I groups, and not significantly different levels in liver enzyme levels (ALT and AST) of V vs C groups. We conclude that the application of the proposed vaccine induced a moderate level of protection and caused a marked defect in parasite fertility. Further studies and natural infection challenges are required to verify its usefulness in the control of ***F. hepatica*** in sheep, as part of an integrated health plan.

611. 360. **THE HUMORAL AND CELLULAR IMMUNE RESPONSE IN CATTLE TO CLOSTRIDIUM CHAUVOEI VACCINATION AND CHALLENGE**

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Vaccination of cattle against *C. chauvoei* is the main strategy to prevent morbidity and mortality caused by this pathogen. Scientific evidence on the underlying immune mechanisms associated with vaccine efficacy is still extremely scarce. Our previous research indicated that specific antibody responses significantly decline 90 days post-vaccination, becoming undetectable after one year, suggesting that antibodies would not be the only correlate of protection. In this context, our study was aimed to identify potential associations between humoral and cellular responses with protection against *in vivo C. chauvoei* challenge, induced by a 9-valent clostridial vaccine. The vaccine conferred complete protection to all vaccinated cattle, which exhibited only mild or no clinical symptoms. Conversely, unvaccinated cattle died due to extremely severe symptoms. The protective response, assessed pre-challenge, was characterized by elevated specific antibodies titers and increased IFN-gamma, TGF-beta, and IL-4 expression. Nevertheless, the extremely severe symptoms observed in unvaccinated animals were associated with low specific antibodies levels, reduced IFN-gamma and TGF-beta expression, and elevated IL-12B mRNA expression. Both pre- and post-challenge, antibody titers and IFN-gamma expression were positively correlated. Additionally, pre-challenge, both antibodies and IFN-gamma positively correlated with TGF-beta, while post-challenge, IL-12B exhibited a negative correlation with both antibodies and IFN-gamma. Our findings suggest an association between vaccine-induced protection and high antibody titers, and elevated IFN-gamma and TGF-beta expression. Moreover, high IL-12B expression, coupled with low antibody titers and reduced IFN-gamma expression, is associated with extremely severe symptoms and poorer prognosis. In summary, these results contribute to a deeper understanding of the immune response elicited by clostridial vaccines, and contribute to improve tools for evaluating vaccine efficacy.

612. 413. **EFFECTS ON GUT BIOLOGY AND PRODUCTIVE PARAMETERS OF THE SUPPLEMENTATION TO GESTATING SOWS AND THEIR PIGLETS WITH KLUYVEROMYCES MARXIANUS PROBIOTIC YEASTS**

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Weaning in porcine production is an abrupt event that stresses animals having an important impact on gut biology. This may affect piglet development leading to poorer growth and economic losses. Historically, preventive administration of antibiotics has been used to ameliorate the productive parameters but nowadays this practice is banned because it contributes to antibiotic resistance generation and alternatives are urgently needed. Our group has studied the probiotic yeast *Kluyveromyces marxianus* which has immunomodulatory capacities and convenient biotechnological properties to be grown on a large scale (Romanin et al, 2016; Pendón et al, 2021). To test the efficacy of this yeast in preventing post-weaning stress, we conducted an experiment where sows (n=8) were supplemented with 10^9 CFU per kg of food from 20 days before the birth of the piglets till the weaning. Then, the piglets were supplemented with $2x10^9$ CFU per kg of food for 20 days more. A control group of sows (n=8) and their piglets not supplemented with the yeast was included. Productive parameters were registered from birth to 70 days of age. Five days post-weaning 5 animals from each group were euthanized; small intestines and blood were taken for histopathology analysis and aminoacid analysis respectively. Intestinal content was sampled to determine the main bacterial groups by qPCR. Piglets born from supplemented sows (n=132) weighed more than the controls (n=130; p<0,01). During nursing, there were no differences among groups but two months after birth the supplemented group weighted 10% more than the control group (p<0,01). We found an increase in citrullinemia and in the small intestine mucosal thickness in supplemented piglets post-weaning indicating better tolerance to post-weaning stress. Our results suggest that *Kluyveromyces marxianus* could be a good candidate as supplementation although deeper characterization of the effects is needed.

613. 446. **PRELIMINARY EVALUATION OF VACCINES DESIGNED AGAINST MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS: IMMUNE RESPONSE AND PROTECTION IN CHALLENGED MICE**

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Paratuberculosis is a chronic and progressive granulomatous enteritis affecting ruminants, caused by ***Mycobacterium avium*** subsp. ***paratuberculosis*** (Map). Our aim was to evaluate two vaccine candidates: 1) Map B mimotopes expressed in M13mp19 bacteriophages (phage), selected by biopanning, and 2) ***in silico*** selected Map T epitopes combined in a chimeric protein expressed in ***E. coli***. Three experimental groups (n=5) received: 10^{12} PFU phages without adjuvant (E1), 30 µg chimeric protein, emulsified in incomplete Freund's adjuvant (E2) and mixed 30 µg chimeric protein + 10^{12} PFU phages, without adjuvant (E3). The control groups (n = 3) received: Map lysate (C1), ***E. coli*** lysate (C2), unselected phage (C3), and PBS (C4). 21 days after the second immunization, blood samples were taken and mice were intraperitoneally challenged with 10^9 CFU of Map. 60 days post-challenge euthanasia was performed, and blood and tissue samples were taken for serology and bacterial culture/histopathology, respectively. (CICUAL-FCV UBA N°: 2022/15). When we measured antibodies directed against Map prior challenge by ELISA, we found that 13/15 animals from the experimental groups responded with O.D. values varying between 0.151 and 1.996 (O.D. for the control groups X + 2 D.S. = 0.100). However, these values did not correlate with survival. No statistical differences were found between IgG subclasses in E1, whilst E2 and E3 showed slightly higher levels of IgG2A and IgG3 ***vs.*** IgG1 and IgG2B (Kruskal-Wallis P<0.05). When culturing intestine samples, only E1 and C1 groups showed a significantly lower bacterial load than the other challenged groups (ANOVA, P < 0.05). Interestingly both groups showed 100% survival, while vaccinated groups E2 and E3 showed 60% and 20%, respectively. The results obtained so far indicate that the phage vaccine evaluated could confer protection in the mouse model and would be a candidate to consider in future evaluations.

614. 459. **BIOPRINTING OF ANTIGENS ENCAPSULATED IN MICROSPHERES US-**

ING ALGINATE AS A BIOGEL

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The advancement of techniques based on 3D bioprinting through various methods, such as encapsulation and controlled drug release, for exploration in the field of immunology. Microencapsulation is being explored as a promising method for antigen controlled release. Furthermore, poly (D, L-lactide-co-glycolide) (PLGA) microspheres (MS) have been successfully applied as efficient drug encapsulation systems. Alginate, a low-cost biomaterial, has demonstrated good bioprinting capability and excellent biocompatibility in hydrogel form. The combination of these developments could be a promising strategy for vaccine production. The objective of this work is to develop a 3D bioprinted vaccine against the disease caused by *Mycobacterium avium* subsp. *paratuberculosis*, that affects cattle, causing gastrointestinal disorders and significant economic losses. MS containing LAM and ovalbumin (OVA) as model protein were synthesized using the double emulsion water/oil/water (W/O/W) method with solvent evaporation. The encapsulation efficiency (EE) was evaluated by quantifying the concentration of antigens using the phenol-sulfuric acid and Bradford techniques respectively. Once the double emulsion was prepared, it was bioprinted using alginate 1% alginate and 3% gelatin mixture at 37°C, in combination with 2 µl of MS. The resulting microparticles were observed under an optical microscope for morphological characterization, and their diameter was measured using ImageJ software. The synthesized MS were homogeneous, with an average diameter of 46.62 microns. After bioprinted, the MS were observed intertwined within the alginate scaffold under an optical microscope.The encapsulation efficiency (EE) was calculated as 95% for LAM and 28.8% for OVA. Despite the challenge of achieving high EE for OVA, simultaneous encapsulation of the antigens was successful. Both antigens could be processed simultaneously, and the subsequent bioprinting process did not affect the morphology of the MS. Further development will involve protocol modifications to improve the EE of both bioprinted antigens for use as an immunogen to evaluate the induced immune response.

615. 513. **CROSS-RECOGNITION OF THE GLYCINE-RICH PROTEIN RMGRP BY SERA OF DIFFERENT MAMMALIAN HOSTS INFESTED WITH THE TICKS RHIPICEPHALUS MICROPLUS OR R. BURSA**

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Ticks comprise multiple species of economic importance. The ticks *Rhipicephalus microplus* and *R. bursa* causes billions of dollars of losses by affecting different animal protein food chains, by directly affecting commercial herds as well as representing important disease vectors. Furthermore, chemical treatment is the main resource nowadays to control populations of these ticks, raising concerns about the impact on the environment and human and animal health. The relationship of ticks and their mammalian hosts depends on saliva components that may modulate the immune and hemostatic systems. Tick saliva contain several glycine-rich proteins (GRPs) that seems to perform diverse roles in these parasites. In special, the GRP RmGRP was characterized as involved in embryogenesis of *R. microplus* and showed to be recognized by infested bovines. Here we show that the recombinant form of RmGRP is also recognized by sera of ovines infested by another tick of the Rhipicephalus genus – R. bursa. Recombinant RmGRP was produced in a procaryotic system and purified using a cobalt metal affinity chromatography resin. SDS-PAGE and Western-blot analyses were performed to ensure specificity and integrity of the purified protein. ELISA analyses were performed in order to compare the recognition levels of sera from different ovines naturally infested with R. bursa from Portugal. Results obtained showed significant recognition of all sera from infested animals, indicating that RmGRP is highly antigenic both in bovines and ovines, and from monoxenic and heteroxenic tick species. Importance of the results concerning the development of broad-acting vaccines, possible immunodominance and redundancy in salivary proteins roles is discussed. Financed by: CNPq, CAPES, FAPERGS, PUCRS and INCT-Entomologia Molecular.

616. 527. SPECIFIC HUMORAL IMMUNITY OF A SOCIAL RODENT, THE CASE OF WILD CAVY (CAVIA APEREA)

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The diversity of roles that antibodies play in resistance to parasites emphasizes their importance in regulating the host-parasite interaction in natural systems. The goal of this work is to evaluate the production of specific antibodies and assess their variability with intrinsic characteristics of the wild cavy (sex, body condition and reproductive status). In order to evaluate the specific immune response in cavies in semi-natural conditions: 107 individuals were kept in four enclosures (0.125 ha), of which 25 adults (females 17; males 9) were immunized at weeks 35 and 37 (from the beginning of maintenance in the closures) with bovine serum albumin (BSA) suspended in aluminum hydroxide. Four recaptures were carried out during a month, in which morphometric variables (mass, body condition), blood sample and reproductive status were taken from each recaptured individual. Serum samples (n=94; females 60/males 34) were evaluated by indirect Elisa. ASB is a T-dependent antigen, which allowed evaluating the investment in specific immunity. A peroxidase-conjugated antibody designed and produced in our laboratory was used. A mixed linear model was applied in order to analyze the results. Levels of anti-albumin antibodies after inoculation increased in all individuals, but were higher in females compared to males. However, this difference depended on body condition: when condition of males´condition was better, the difference between both sexes was smaller. Investment in specific immunity would depend on physiological demands: females maximize their fitness through greater investment in immune responses, thus favoring more offspring, while males maximize their fitness by betting on mating success (Bateman's principle). In this way, females would invest more in specific humoral immunity than males as a means of survival since their reproductive effort is much greater.

617. 528. CPG-ODN FORMULATED WITH A COA-ASC16 NANOSTRUCTURE AS ADJUVANT FOR EXPERIMENTAL ANTI-BOTHROPIC SERUM PRODUCTION.

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The only treatment for snakebite is antivenom, typically produced by immunizing animals such as horses with snake venom, using Freund's adjuvant. However, Freund's adjuvant is associated with local and systemic damage, compromising the welfare of producer animals. To mitigate the adverse effects associated with mineral oil-based adjuvants, new formulations are being explored. Previous studies demonstrated that the CpG-ODN/Coa-ASC16 adjuvant combined with *C.d. terrificus* venom induces neutralizing antibodies with minimal local reactions, suggesting potential for further exploration with Bothropic venom. We formulated *Bothrops diporus* venom (B.dV) with CpG-ODN/Coa-ASC16 (B.dV/CpG-ODN/Coa-ASC16). This formulation elicited a robust immune response with high antibody titers ($5.12x10^{04}$) and reduced local histological damage in mice. Given these promising results, the present study aimed to evaluate the avidity and neutralizing activity of the experimental antivenom produced from the B.dV/CpG-ODN/Coa-ASC16 formulation against B.dV. BALB/c mice were subcutaneously immunized on days 0, 15, and 30 with 7-30 µg of B.dV, either formulated with CpG-ODN/Coa-ASC16 or Freund's adjuvant, and sera were collected on day 45. Antibody avidity was assessed using a chaotropic agent (KSCN), and the neutralizing activity was evaluated through in vitro tests. On day 45, the anti-B.dV/CpG-ODN/Coa- ASC16 sera exhibited an avidity index of 3.9 M, indicating significantly higher avidity compared to the B.dV/Freund formulation ($p<0.05$). Both experimental sera also effectively neutralized proteolytic, indirect hemolytic, and coagulant activities in vitro. In summary, the CpG-ODN/Coa-ASC16 adjuvant combined with Bothropic venom rep-

resents a promising and innovative formulation, offering not only a strong immunogenic response while reducing adverse effects on the producer animals, thereby enhancing their welfare.

618. 615. **ANALYSIS OF IMMUNOPROTECTION IN SHEEP AGAINST FASCIOLA HEPATICA THROUGH THE COMBINATION OF FHLAP1/FHLAP2 AND SAPONIN-BASED NANOPARTICLES**

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Proteases in parasitic helminths are involved in the processing of maturation, activation, and/or degradation of substrates, making them the promising vaccine targets. Leucine aminopeptidases (LAPs) part of the M17 Zn-metalloprotease family cleaves leucine residues at the N-terminal ends of proteins and peptides, playing a role in substrate processing and activation. Herein, we tried a vaccine formulation using a two Leucine aminopeptidases recombinant, co-administered with ISCOM-matrices nanoparticles Quillaja brasiliensis saponin-based adjuvant (IMXQB). In the present study, we used twenty female Corriedale sheep (six-months-old) which were separated into three groups (n = 10) and each group was accommodated in different rooms. Sheep were immunized twice with parasites antigen FhLAP1/FhLAP2 (100 µg/100 µg) co-adjuvanted with IMX, which was used as control. All formulations were administered subcutaneously on weeks 0 and 4. In week 6, the sheep were orally challenged with 200 metacercariae. The sera were collected at weeks 0, 4, 6, 8, 10, and 18 post-inoculation of the first dose.

Despite the increase in antibody responses in vaccinated groups compared to the control, necropsy results showed no significant differences in liver damage or worm burden among the groups. We also conducted immune profiling using qPCR to analyze gene expression of cytokines (GAPDH, IFN-γ, TNF-α, IL-1β, IL-10, TGF-β, IL-2, IL-17, IL-4, IL-5 and FoxP3), providing insight into the humoral and cellular responses. This research offers important data for the future development of vaccines in livestock, a field with limited experimental models. Additionally, the vaccine formulations use a natural adjuvant that can be sourced sustainably, supporting a one-health approach by avoiding environmental harm.

619. 637. **G PROTEIN-COUPLED ESTROGEN RECEPTOR IMMUNOLABELING AND ESTROGEN CONCENTRATION DURING PORCINE GESTATION**

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The G protein-coupled estrogen receptor (GPER) is found in various estrogen-responsive tissues, such as uterus, ovaries, and mammary glands, and is widely distributed in cardiovascular, immune, and skeletal systems. GPER is involved in the regulation of cellular functions, such as proliferation, metastasis, differentiation, and apoptosis. The aim was to detect the presence of GPER during pregnancy in sows and establish its relationship with estrogen concentration (Es). Crossbred sows were used at different stages of gestation: 5 days of gestation (dg), 15-17 dg, 30-35 dg and 60-70 dg, and non-pregnant sows in follicular (NGF) and luteal (NGL) phases. The presence of GPER was determined by indirect immunoperoxidase, the results were expressed evaluating the intensity of the brown coloration detected. Es was measured by chemiluminescence in sera and placental extracts. In maternal epithelium, GPER showed an increase from 5 dg, remained between 17-20 dg and decreased towards 60-70 dg; while in glandular epithelium it was moderate between 17-20 dg and reduced towards 60-70 dg. In trophoblast, the expression was moderate in the analyzed periods. Immunolabeling in maternal vessels was moderate in all periods, except between 17-20 dg, where it was mild. In fetal vessels, a mark of 30-35 dg was determined, being mild at 60-70 dg. Serum estrogen levels remained constant between 5 and 30 dg, with a significant increase at 70 dg, similar to the values in NGF. In maternal placental extracts,

Es increased up to 30 dg and then decreased to periimplantation levels. In fetal placental extracts, concentration was high at 17 dg, decreasing at 30-35 dg and remaining stable at 60-70 dg. The presence of Es during gestation together with the expression of GPER in different placental structures suggests that they could work together with the classic Es receptors to comprehensively regulate uterine physiology and achieve a successful pregnancy

620. 638. **IMMUNOLOCALIZATION OF INTEGRIN ALPHA V BETA 3 AND ITS LIGANDS DURING SOW PLACENTATION**

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The precise function of avb3 and its ligands in sow gestation is not yet known, but may include cell signaling, adhesion, or regulation of innate immunity during placentation. The objective of this work was to determine the expression of the integrin avb3 and its ligands vitronectin, fibrinogen, osteopontin and fibronectin in porcine placentas. Uteri from non-pregnant sows and placentas of 17, 30, 60, 70 and 114 days of gestation (dg) were collected. The determination of the molecules was carried out by indirect immunoperoxidase. The results were expressed in a semi-qualitative way based on the detected coloration, determining that: (-)= negative, (+)= slight positivity, (++)= moderate positivity and (+++)= strong positivity. The presence of avb3 was observed in endometrial epithelium and trophoblast from 17 dg (++) increasing to 60 dg (+++) decreasing towards the end of gestation; vitronectin, osteopontin and fibronectin had a similar behavior. In maternal vessels, the presence of avb3 was moderate at 17 dg (++), decreasing in the following periods, but not its ligands, which showed high expression during almost the entire pregnancy. In glands, avb3 was not observed but vitronectin and osteopontin were. The immunolocalization of the avb3 integrin and its ligands in the feto-embryonic-maternal interface, would suggest an active participation in the formation and development of the placental structure. In endometrium, in blood vessels the low immunostaining of avb3 and the high expression of its ligands would indicate the participation of other integrins during placentation; and the glandular architecture would be given in principle by the interaction of vitronectin and osteopontin with other intervening molecules; whereas fibrinogen and fibronectin would not participate.

621. 639. **PROFILE OF INTERLEUKINS, PROGESTERONE AND ASYMMETRIC ANTIBODIES IN MID/LATE GESTATION OF THE SOW.**

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Molecules such as interleukins (ILs), progesterone (P4) and asymmetric antibodies (AAs) play a critical and dynamic role during gestation, regulating the immunological conditions of pregnancy. Concentration of ILs, P4 and AAs in serum, uterus from non-pregnant sows and placental extracts from pregnant sows of mid (60-80 days) and late (85-114 days) gestation were analyzed. Regarding the maternal component, in serum, IL-10, IL-4 and P4 are elevated in mid-gestation. In the late stage, serum IL-10 and IL-4 levels increase further, while P4 levels decrease. IL-6, IL-15 and AAs remain constant throughout gestation. In the maternal placenta, IL-6, IL-4 and AAs increase significantly in mid-gestation. During this stage, IL-15 decreases compared to its values in non-pregnant endometrium. On the other hand, IL-10 and P4 remain stable at basal levels. In late gestation, IL-10, IL-6 and P4 remain stable at basal values and IL-4 and AAs decrease significantly. In the fetal placenta, IL-6, IL-10 and IL-15 levels remain constant at basal levels, while P4 and AAs remain elevated. On the other hand, IL-4 increases in the fetal placenta in mid-gestation and decreases significantly at term. The pattern of cytokines and AAs observed in mid-gestation would reflect immunotolerance, which would allow fetal growth without activating adverse responses, and would modulate the immune response to prevent fetal rejection. In late-gestation, the molecular pattern found, both at systemic and local level, suggests the production of an environment that protects the mother from the proinflammatory environment generated to prepare for the delivery

process. These changes during mid-late gestation in the sow reflect the complexity and precision of immune regulation necessary to ensure a successful gestation; coordinated molecular dialogue that leads to abortion when imbalanced. More studies are needed on the immunology of mid/late gestation in the sow, a species with high fetal losses without a specific cause.

622. 643. EVALUATION AND OPTIMIZATION OF A RECOMBINANT BOVINE TUBERCULOSIS DIAGNOSTIC REAGENT FOR TESTING IN CATTLE

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Bovine tuberculosis (bTB) is a serious infectious disease with major economic impacts due to reduced productivity and costly eradication programs. In Argentina, the standard diagnostic method is the tuberculin skin test using Bovine Purified Protein Derivative (PPD-B). While this method is effective in detecting infected animals, its specificity is compromised by cross-reactivity with Environmental Nontuberculous Mycobacteria. This study aimed to evaluate the biological activity of a recombinant bTB detection reagent (RRbTB), composed of a fusion protein of *Early Secreted Antigenic Target* 6 kDa (ESAT-6) from *Mycobacterium bovis*, and to optimize its use for testing in cattle. The fusion protein and its control without ESAT-6 (RRc) were expressed in *Escherichia coli* and purified by affinity chromatography. The biological activity was assessed through *in vivo* skin tests to detect intradermal reactions (IDR). Four independent assays were conducted in guinea pigs sensitized with inactivated *M. bovis* AN5 strain (n=24). One assay was conducted in cattle (n=12 naturally infected; n=3 infection free). All *in vivo* assays were performed using a Latin square design. RRbTB induced equivalent IDR in guinea pigs compared to standard PPD-B across independent assays, which was confirmed by one-way ANOVA. Using a parallel line assay, it was determined that the relative potency of RRbTB ranged from 110% to 135% compared to standard PPD-B. In cattle, RRbTB did not induce IDR in infection free animals, whereas 8 out of 12 naturally infected cattle exhibited positive responses. In conclusion, RRbTB elicits a reproducible IDR response in guinea pigs that is equivalent to PPD-B. Moreover, the partial IDR response observed in cattle suggests that further optimization is needed. It is expected that dose adjustments and the incorporation of additional *M. bovis* antigens could enhance the response, thus revealing the potential of RRbTB as an alternative diagnostic tool for bTB.

623. 656. IMMUNIZATION WITH ENGINEERED S. AUREUS BETA TOXIN INDUCES FUNCTIONAL ANTIBODIES IN DAIRY HEIFERS

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The *S. aureus* Beta toxin (Btox), a sphingomyelinase causing necrosis of mammary tissues and inflammatory changes, has been used by our group as part of a multicomponent vaccine. However, its recombinant expression is challenging due to inclusion body formation and refolding difficulties. This study aimed to optimize Btox expression in *E. coli* through *in silico* structural modeling and optimization, and evaluated the immune response generated by the obtained variant in dairy heifers. The *S. aureus* Btox structure was predicted using artificial intelligence methods and compared with experimentally resolved homologous structures. *In silico* modeling identified key mutations, predicted to increase stability and solubility and

altering the active site to reduce hemolytic activity of the protein. The designed sequence was cloned in frame to a HisTag into an expression vector for cytoplasmic expression in *E. coli*. After expression, target protein was purified by affinity and size exclusion chromatography, yielding high amounts of soluble monomeric protein. A field trial used two groups of 9-11-month-old heifers (n=5), receiving 3 doses (days 0, 21, 180) of either the recombinant wild-type Btox (formalin inactivated) or the inactive engineered Btox, both with 15% AlOH. A third group served as untreated control. Serum samples collected 10 days after the second and third doses were evaluated for antibody titers by ELISA and inhibition of hemolytic activity *in vitro* using bovine red blood cells. In dairy heifers, the engineered Btox induced specific antibody titers comparable to recombinant wild-type Btox (P=0.451). These antibodies inhibited native wild-type Beta toxin's hemolytic activity *in vitro*, but to a lesser extent than those generated by the recombinant wild-type Btox (P<0.05). Engineered Btox showed significant correlation between antibody titer and hemolytic inhibition at both timepoints (day 31: P=0.012, R=0.954; day 190: P<0.001, R=0.998). These results suggest the engineered Btox as an effective and safer alternative immunogen.

624. 674. **CHITOSAN NANOPARTICLES AS POTENTIAL IMMUNOMODULATORY AGENTS FOR BOVINE MASTITIS PREVENTION**

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Bovine mastitis is an inflammatory disease of the mammary gland tissue, primarily caused by bacterial infections. This condition is highly relevant in the dairy industry due to its impact on animal welfare and the significant economic losses associated with decreased milk yield and quality. Although antibiotic therapies are still the primary strategy for treating bovine mastitis, the increasing concern about antibiotic-resistant pathogens necessitates reduced antimicrobial use, prompting the search for alternative treatments, especially those derived from natural products. Given that dairy cattle with enhanced and balanced immune responses exhibit a lower incidence of the disease, immunostimulant treatments emerge as a promising alternative. Our research focuses on chitosan, a biocompatible, bioactive, and non-toxic polymer with well-documented antimicrobial and immunostimulant properties. Specifically, we developed chitosan nanoparticles prepared using the reverse micelles method, which demonstrated enhanced microbicidal effects against *Staphylococcus aureus* strains isolated from cows with mastitis, compared to the native polymer. When assessing their effects on the immune system, RAW 264.7 macrophages pretreated with chitosan nanoparticles showed increased production of reactive oxygen species after infection with *S. aureus* V329, as measured by flow cytometry using the DCFDA probe. Additionally, treated macrophages exhibited upregulation of pro-inflammatory cytokine mRNA, such as IL-1β and IL-6, as evaluated by qPCR, and increased expression of MHCII, CD86, CD11b, and TLR2 markers, as evidenced by flow cytometry. Importantly, the treatment was non- cytotoxic to both murine macrophages and bovine epithelial cells (MAC-T), as determined by the MTT assay. Overall, our findings highlight the potential of chitosan nanoparticles as a novel therapeutic approach to enhance the immune response of the bovine mammary gland and effectively prevent mastitis development following pathogen infections.

625. 679. **IMMUNOMODULATORY AND ANTIMICROBIAL MODULATION BY 1,25-DIHYDROXYVITAMIN D3 IN BOVINE CELLS: IMPLICATIONS FOR THE TREATMENT OF BOVINE MASTITIS**

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The active metabolite of vitamin D3, 1,25-dihydroxyvitamin D3 (or calcitriol), regulates gene expression in various cell types by modulating proliferation, differentiation and immune response. In order to explore these mechanisms in cattle, the production of proteins and other immune mediators was evaluated in bovine mammary epithelial cells (MAC-T) and bovine macrophages (BoMac) after calcitriol treatment. Reactive oxygen species (ROS) production was analyzed using fluorescence microscopy and proteomic analysis of

conditioned media from MAC-T (MCE) and Bo-Mac (MCM) cells was performed by mass spectrometry. Subsequently, the effect of these media on preformed biofilms of *Staphylococcus* spp. isolated from bovine mastitis cases was evaluated. Biofilm structure and biomass were assessed using confocal microscopy and the crystal violet technique, respectively. Statistical analysis was conducted using ANOVA/Bonferroni tests. Calcitriol induced ROS production and promoted the secretion of immunomodulatory and antimicrobial proteins in both MCE and MCM. Furthermore, conditioned media from calcitriol-treated cells reduced biofilm biomass and caused bacterial death. These findings suggest that calcitriol holds significant potential as a preventive or therapeutic treatment for bovine mastitis.

626. 724. **NEW-GENERATION VACCINES AGAINST FOOT-AND-MOUTH DISEASE VIRUS: STRATEGIES TO ENHANCE THE IMMUNOGENICITY OF RECOMBINANT EMPTY CAPSIDS**

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Foot-and-mouth disease (FMD) is a highly contagious disease that affects economically important animals. It is caused by a picornavirus, the foot-and-mouth disease virus (FMDV). Current vaccines are based on inactivated FMDV but they have significant limitations, including the need to produce live viruses on a large scale. Virus -like particles (VLPs) have been shown to be safe, with high antigenic capacity; however, they require the use of adjuvants due to their low immunogenicity. This study aims to explore biotechnological strategies for rationally designing VLP-based vaccines. These strategies include immunomodulation with baculovirus, the complement of cytotoxic cellular responses with recombinant baculoviruses that carry in their nucleocapsids FMDV-derived antigens (Ag) and Ag-edited capsids that provide cross-protection against different serotypes of the virus. To achieve this, the recombinant baculoviruses AcVP1cap, AcP1-2A-3Cfs, and AcP1Aless-2A-3Cfs were constructed. AcVP1cap carries the FMDV capsid protein VP1, whereas AcP1-2A-3Cfs and AcP1Aless-2A-3Cfs yield conventional empty capsids and empty capsids lacking the immunodominant antigenic Site A of FMDV A/Arg/2001, respectively. Infectious baculovirus titers were calculated in Sf9-GFP cells using an endpoint dilution assay and converted to PFU/ml. VLPs were obtained at 6 days post-infection from *Rachiplusia nu* pupae inoculated with 1 x 10^6 PFU. The purification method involved sedimentation on an 18-45% sucrose gradient and ultracentrifugation. The VLPs were detected and quantified using Western blot and enzyme-linked immunosorbent assay (ELISA), respectively. The obtained yield from this system was 1.19 µg/g pupa. Finally, the proper three-dimensional conformation and particle size were studied by transmission electron microscopy (TEM) and dynamic light scattering (DLS). *In vivo* experiments are underway to evaluate the immunogenicity of the VLPs in the proposed formulations. In terms of cost, performance, efficacy, and safety, this work offers good prospects for vaccine design and production

627. 331. **IMMUNOLOGICAL EVALUATION OF ANTIVENOMS FOR BOTHROPS ALTERNATUS ENVENOMATION USING VENOMIC AND ANTIVENOMIC APPROACH**

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Bothrops alternatus is a highly venomous snake found in South America. Currently, anti-serum therapy is the only treatment for snakebites, and animals are used in the preclinical analysis of antivenoms. However, in vitro techniques are being developed to reduce or eliminate the need for animal use. In this study, we evaluated two antivenoms used in the treatment of *B. alternatus* bites in Argentina. Using a venomic approach, we performed the proteomic characterization of *B. alternatus* venom. Two antivenoms, anti-BaINPB® and anti-BaBIOL®, were tested for their immunoreactivity against venom components. The venom comprises toxins from eleven protein families. The main identified families included PIII-SVMP (27.32%), PII-SVMP (12.33%), D-SVMP (10.26%), SVSP (8.80%), PLA_2 (6.85%), BBP/C-NP (4.85%), CRVP (4.87%), CTL (6.80%), LAAO (4.14%), SVGF (1.90%), VPA (1.64%), PDE (0.07%), and an unidentified percentage (11.64%). Antivenomic analysis showed that both antivenoms recognized all venom proteins but had low affinity for small peptides like BPP. The BIOL® antivenom demonstrated a binding capacity of 57 mg/g $F(ab')_2$, while the INPB® antivenom showed a significantly higher binding capacity of 149 mg/g $F(ab')_2$. Focusing the analysis on the immunorecognition of antibodies against specific proteins, a competition is observed with the immunorecognition of a disintegrin-like protein and PIII-SVMP, which is not observed when the analysis is performed on a PII-SVMP and a disintegrin fragment. The anti-BaINPB® antivenom shows greater differential immunorecognition of the PLA_2 proteoforms compared to the anti-BaBIOL® antivenom. This difference suggests variations in their efficacy against different components of *B. alternatus* venom. These results align with in vivo studies, where the antivenom potency was 68.28 mg/g $F(ab')_2$ and 129.38 mg/g $F(ab')_2$ for BIOL® and INPB®, respectively. This study highlights the value of third-generation antivenomics in characterizing antivenoms used against *B. alternatus* envenomation and confirms the effectiveness of these two antivenoms in treating snakebites caused *by B. alternatus*.

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