INTERNATIONAL ASSOCIATION OF INFLAMMATION SOCIETIES

Final Program

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16TH WORLD CONGRESS ON INFLAMATION

We have the pleasure to welcome you to the WCI2024 that is taking place in Québec City from July 21 to 24, 2024.

The theme of this meeting is: *Inflammation in Health & Disease: A Balancing Act*

Highlighted themes include: bones and skin, immunometabolism, infectious diseases, lungs, microbiota, mucosal inflammation, neuro-inflammation, pain, resolution of inflammation, trained immunity, and much more!

The Congress brings together researchers, clinicians, industry representatives, advocacy groups, and decision-makers across the field of inflammation. WCI2024 includes the latest developments in basic, translational and clinical research on inflammation, including cell biology, signalling, genetic, inflammatory/autoimmune diseases, new biomarkers and new therapeutic approaches for human diseases.

The biennial WCI Congress, which brings together a number of Scientific Societies within the International Association of Inflammation Societies and other Scientific Societies who attend the Congress, represents a key opportunity to share knowledge across borders and professional disciplines as we work towards a common goal of improving the understanding and therapeutic control of inflammatory diseases. The congress enhances the international community's knowledge base and provides new opportunities for international collaboration.

Québec City is the only fortified city in North America and the oldest city on the continent north of the Mexican border. Founded in 1608, the city is famous for its European character and magnificent landscapes, which make it a highly valued and internationally recognized tourist destination. Québec City also proudly appears on UNESCO's World Heritage List.

We are most honored to welcome you for WCI2024 in the beautiful city of Quebec!

-Your organizing committee

Program - WCI 2024

Opening Conference: Unlocking the power of trained immunity in pulmonary infections

21/07/2024 17:30-18:30 Room: 200A

17h30 Unlocking the power of trained immunity in pulmonary infections

Keynote: Homeostasis: Just a level of inflammation

22/07/2024 08:30-09:30 Room: 200A

8h30 Homeostasis: Just a level of inflammation

Symposium sponsored by the GREMI, French Society of Inflammation and Ambiotis: Novel Points of Control of Lung Inflammation

22/07/2024 09:30-10:45 Room: 200A

9h30 Effect of CFTR modulators on lung inflammation and neutrophil functions in cystic fibrosis

9h45 Neonatal cross-talk of alveolar macrophages and neutrophils regulate lung inflammation

10h00 Metabolic control of virus-induced lung inflammation
Si-Tahar, Mustapha

10h15 Specialized pro-resolving mediators in respiratory diseases

Symposium USA-1: Translational resolution of chronic inflammation
Li, Liwu

22/07/2024 09:30-10:45 Room: 301B

9h30 Systems approach in dampening lung inflammation

9h45 Systems analyses at single cell level for cardiovascular inflammation

10h00 The yin and yang of mTOR and lysosomal nutrient sensing in macrophages and atherosclerosis

10h15 Rejuvenation of innate homeostasis in the treatment of disease

Program - WCI 2024

Lung 1
Norel, Xavier

22/07/2024 11:00-12:00 Room: 200A

11h00 Identification of a novel macrophage subset involved in pulmonary fibrosis by intravital imaging techniques
Kikuta, Junichi - Suzuki, Akio - Ishii, Masaru

11h15 Targeting RIPK3-mediated epithelial cell necroptosis protects against RSV infection
Porto, Barbara - Cerato, Julia - Serda, Maria - Duan, Wenming - Moraes, Theo - Coombs, Kevin

11h30 Eosinophil phenotypes are functionally regulated by resolvin D2 during allergic lung inflammation
Bruggemann, Thayse - Peh, Hong Yong - Tavares, Luciana P. - Nijmeh, Julie - Shay, Ashley E. - Rezende, Rafael M. - Lanser, Toby B. - Serhan, Charles N. - Levy, Bruce D.

11h45 Role of PGE2 on human bronchial and vascular tone: comparison in lungs with and without COPD
Merheb, Gaelle

NETs
Bourgoin, Sylvain

22/07/2024 11:00-12:00 Room: 301B

11h00 Product impurity takes its toll: The actual ability of Toll-like receptors to generate neutrophil extracellular traps (NETs)
de Carvalho Oliveira, Vanessa - Tshivuadi Mosha, Hugo

11h15 Low density neutrophils and neutrophil extracellular traps (NETs) are new inflammatory players in heart failure
Sirois, Martin G. - Dumont, Benjamin L. - Neagoe, Paul-Eduard - Charles, Elcha - Villeneuve, Louis - Ninni, Sandro - Tardif, Jean-Claude - Räkel, Agnès - White, Michel

Stimulating the resolution of inflammation
Li, Liwu

22/07/2024 11:00-12:00 Room: 301A

11h00 The BiST of Burden: Harnessing biased STING agonists to enhance the resolution of inflammation and limit tissue fibrosis
Ariel, Amiram - Ben Jashar, Nofar - Saqib, Uzma - Schif-Zuck, Sagie

11h15 PEPITEM switches off the production of pro-inflammatory mediators to limit leukocyte trafficking into the inflamed joint
Wahid, Mussarat - Abudu, Oladimeji - Kemble, Samuel - Mahony, Chris - Saviano, Anella - Schettino, Anna - Marigliano, Noemi - Urbanowski, Alyssa - Filer, Andrew - Raza, Karim - Jilani Iqbal, Asif - Maione, Francesco -

11h30 Fpr1/Fpr2-Based Resolution Pharmacology Attenuates Cardiomyopathy in Inflammatory Arthritis
Chen, Jianmin - Bu, Weifeng - Cooper, Dianne - Norling, Lucy - A. Lupisella, John - A. Garcia, Ricardo - Perretti, Mauro

11h45 Ameliorating Cancer Cachexia by Inhibition of Soluble Epoxide Hydrolase
Bayer, Rachel - Virani, Sarina - Quinlivan, Katherine - Gillespie, Michael - Smith, Keira - Yang, Haixia - Hammock, Bruce

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Systemic inflammation and intervention 1
Teixeira, Mauro

22/07/2024 11:00-12:00 Room: 303B

11h00 Role of IRG1 in Regulating Inflammatory Signaling
Papareddy, Praveen

11h15 Thrombopoietin-dependent megakaryopoiesis fuels thromboinflammation and worsens antibody-mediated chronic renal microvascular injury
Douté, Mélodie - Sannier, Aurélie - Even, Guillaume - Tran, Thi-Thu - Gaston, Ahn-Tu - Delbosc, Sandrine - Loyau, Stéphane - Bruneval, Patrick - Witko-Sarsat, Véronique - Mouthon, Luc - Nicoletti, Antonino - Caligiuri, Giuseppina -

11h30 Relationship of serum levels of inflammatory marker endocan with apoptosis and severity of atherosclerotic coronary artery lesions
Kubyshkin, Anatolii - Zakharyan, Elena

11h45 A Leak Along the Line: Chronic Inflammation Impairs Collecting Lymphatic Junction Integrity
Keane, Keith - Stephens, Matthew - von der Weid, Pierre-Yves

Late-breaking
Flamand, Nicolas

22/07/2024 11:00-12:00 Room: 303A

11h00 Resolvins enhance immunotherapy to induce Fanconi anemia tumor regression via inflammation resolution
Southan, Franciska - Quinlivan, Katherine - Yang, Haixia - Bielenberg, Diane - Wells, Susanne - Serhan, Charles - Panigrahy, Dipak

11h15 Discoidin domain receptor 1 (DDR1) contributes to the development of arthritis by promoting the migration of pathogenic Th17 cells through collagen
Hamoudi, Chakib - Toghi, Mehdi - Aoudjit, Fawzi

11h30 Unlocking the Pharmaceutical Potential of Specialized Pro-resolving Mediators by Chemical Synthesis of Simplified Structural Mimics
Maltais, Rene - Marette, André - Sancéau, Jean-Yves - Poirier, Donald - Boivin, Guy

11h45 Neutrophil extracellular traps trigger polyclonal B lymphocyte activation independently of antigen specificity toward a pro-inflammatory response
Haidar Ahmad, Ahmad - Batignes, Maxime - Lemeiter, Delphine - Melbouci, Dyhia - Semerano, Luca - Decker, Patrice

Plenary lecture: Decoding the resolution response: Resolvin & cys-SPM functions in inflammation novel molecular links to tissue regeneration and wound healing

22/07/2024 12:45-13:45 Room: 200A

12h45 Decoding the resolution response: Resolvin & cys-SPM functions in inflammation novel molecular links to tissue regeneration and wound healing

Symposium Brazil - GREMI (France) sponsored by Solutex: Lipid mediators for resolution and beyond

22/07/2024 13:45-15:00 Room: 200A

13h45 SPMs in atherosclerosis and aging

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14h00 Maresin and SPMs in human arteries and heart, interactions with PGE2 pathway

14h15 Novel molecules and mechanisms resolving inflammation, suppressing fibrosis

14h30 Abnormal resolution of inflammation in Cystic Fibrosis: A role for Specialized Proresolving Mediators

Symposium Canada-1: New targets in mucosal inflammation
Rossi, Adriano

22/07/2024 13:45-15:00 **Room: 301B**

13h45 Investigating the role of ELA2, an epithelial elastase, in impaired muscosal healing in chronic inflammatory bowel diseases: Insights and therapeutic prospects

14h00 Beyond the gluten-free diet: Therapeutic targets in celiac disease

14h15 Novel approaches to target initiation, propagation and resolution of mucosal inflammation

Plenary Talk: Osteoimmunology

22/07/2024 15:15-16:15 **Room: 200A**

15h15 Osteoimmunology

Symposium Japan: Inflammatory diseases involving bones, skin and beyond: Systemic and systematic approaches

22/07/2024 16:15-17:30 **Room: 200A**

16h15 Understanding inflammatory diseases involving bone, skin and beyond: An overview

16h30 Immune and inflammatory regulation of bone and inter-organ crosstalk

16h45 Imaging of inflammatory effector cell responses in bone, skin and beyond

17h00 Inter-organ crosstalk view of skin inflammatory disorders

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17h15 Key immunological pathways in systemic autoimmune disease revealed by functional genome analysis

Symposium organized by Italian Society of Pharmacology (SIF): Microglia as a new target for therapeutic approach to neuroinflammation-based brain disorders

22/07/2024 16:15-17:30 Room: 301B

16h15 The FGF/FGFR system in the microglial activation caused by neuroinflammatory brain disorders

16h30 Role of the microglia cells in models of nociplastic pain

16h45 Glia reactivity and neuroinflammation in neurodegeneration: From cellular changes to possible therapeutic approaches

17h00 ADAM10 at the crossroad of synaptic failure and inflammation in Alzheimer's Disease

Plenary lecture: Taming inflammation to treat infection

23/07/2024 08:30-09:30 Room: 200A

8h30 Taming inflammation to treat infection

Symposium Brazil-2: Inflammatory responses in infectious diseases

Teixeira, Mauro - Queiroz Cunha, Fernando

23/07/2024 09:30-10:45 Room: 200A

9h30 COVID-19: Involvement of NET in organ lesions

9h45 Infection diseases: Role of thromboinflammation in infection diseases

10h00 Infection diseases: Participation of inflammasome in immunopathology of infections

Symposium USA-2: Innate immune memory dynamics in health and disease

Li, Liwu

23/07/2024 09:30-10:45 Room: 301B

9h30 Neutrophil-neutrophil communication, swarming, and anti-fungal actions: A rapidly changing paradigm in the lab and in the clinic

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9h45 Stepwise transcriptional reprogramming directs neutrophil trafficking and function in inflamed lungs

10h00 Finding and pushing the limits of macrophage efferocytosis

10h15 TBA

Symposium Organized by CIHR: Evidence to Impact: CIHR Inflammation Teams in Translational Research

23/07/2024 09:30-10:45 Room: 303B

9h30 Therapeutically targeting vascular inflammation in the setting of metabolic stress: Translational opportunities & challenges

9h37 Regulatory NK cells as Therapy for Chronic Graft-versus-Host Disease and Autoimmunity

9h44 Targeting cerebral small vessel disease by inhibition of the soluble epoxide hydrolase

9h51 Plasma endocannabinoidome lipids and fecal microbiota composition in people with HIV under antiretroviral therapy with diagnosed subclinical coronary artery disease: results of the Canadian HIV and Aging Cohort (CTNPT 043 study)

9h58 Treg/Th17 balance at mucosal surfaces in HIV and IBD

10h05 Moderated Panel Discussion

Lung 2

Porto, Barbara

23/07/2024 11:00-12:00 Room: 200A

11h00 Transforming Growth Factor Beta Reduces Neutrophil Motility by Promoting Formation of Neutrophil Clusters in Lung Capillaries
Li, Ziyi - Kwak, Ashley - Groleau, Marc - Kulle, Amelia - Belur, Vaishnav - Thanabalasuriar, Ajitha

11h15 EP 80317, a CD36 ligand, attenuates remote lung injury after transient hind limb ischaemia in mice
Elimam, Hanan - Gauvin, Jade - Al-Hawat, Marie-Lynn - Huynh, David N. - Ménard, Liliane - Ong, Huy - Marleau, Sylvie

11h30 Sex-specific impact of early life stress on lung inflammatory response in adults
Bouchard, Karine - Patoine, Dany - Roy, Joanny - Fournier, Stéphanie - Marsolais, David - Kinkead, Richard - Lauzon-Joset, Jean-François

11h45 Unlocking the Potential of Omega-3 Fatty Acids: Modulation of Vascular Tone in Pulmonary Hypertension
Badji, Hichem - Merheb, Gaelle - Renson, Louis - Longrois, Dan - Norel, Xavier

Immunometabolism
Sweet, Matthew

23/07/2024 11:00-12:00 Room: 301B

11h00 Modulation of Immunometabolism Via NLRX1 Or PLXDC2: Novel Bimodal Mechanisms for the Treatment of Inflammatory Bowel Diseases
Danese, Silvio - Colombel, Jean-Frederic - Rieder, Florian - Peyrin-Biroulet, Laurent - Siegmund, Britta - Vermeire, Severine - Dubinsky, Marla - Schreiber, Stefan - Yarur, Andres - Panaccione, Remo - Feagan, Brian - Mosig,

11h15 Suppression of Adipocyte ABHD6 in Mice Promotes Healthy Expansion of Adipose Tissue in Obesity
Poursharifi, Pegah - Attané, Camille - Klein, Laura-Lee - Calce, Sara-Ivana - Mootoosamy, Covida - Shea, Jonathan - Chenier, Isabelle - Ghosh, Anindya - Schmitt, Clemence - Lussier, Roxane - Al-Mass, Anfal - Leung, Yat Hei -

11h30 Investigating the role of endocannabinoid and immune system in the small intestine of severe obese subjects with type-2 diabetes
Rakotoarivelo, Volatiana - Allam-Ndoul, Bénedicte - Mayer, Thomas Z. - Biertho, Laurent - Flamand, Nicolas - Di Marzo, Vincenzo - Veilleux, Alain

Redox
Witko-Sarsat, Véronique

23/07/2024 11:00-12:00 Room: 301A

11h00 Targeting Cysteine Residues as modulators of STAT1 Activity: Implications for Infectious and Autoimmune Disorders
Paz-Trejo, Cynthia - Fortin, Audray - Harrison, Alex - Caron, Elise - Zamorano Cuervo, Natalia - Grajales, Zayd - Chartier, Stéfany - Grandvuax, Nathalie

11h15 The anti-atherosclerotic agent MPE-298 causes alternate trafficking of the cluster of differentiation 36 receptor (CD36) in macrophages
Lê, Catherine - Mulumba, Mukandila - Schelsohn, Emmanuelle - Lubell, William D. - Marleau, Sylvie - Ong, Huy

11h30 Neutrophils release glycosaminoglycans to form proteoglycofili and NETs
Barroso, Marina - André, Antonin - Skerniskyte, Jurate - Siegwald, Mélina - Debande, Lorine - Paul, Vanessa - Broussaudier, Nathan - Thahouly, Tamou - Ridley, Caroline - Svahn, Isabelle - Bowler, Amber - Rigaud, Stéphane - Len, Kateryna - Gies, Vincent - Metzger, Daniel - Laverny, Gilles - Korganow, Anne-Sophie - Tinevez, Jean-Yves -

11h45 New insights into neutrophil-derived myeloperoxidase driven inflammation
Cartwright, Ian - Zhou, Liheng - Koch, Samuel - Welch, Nichole - Onyiah, Joseph - Steiner, Calen - Colgan, Sean

Systemic inflammation and intervention 2
Buret, André

23/07/2024 11:00-12:00 Room: 303B

11h00 Skin innate immune response to Usutu virus infection
Bodet, Charles - Vouillon, Axelle - Lévêque, Nicolas - Garcia, Magali

11h15 Molecular characterisation based on current geneset databases may not sufficiently define inflammatory processes in conditions with pronounced inflammation: results from the X-HiDE consortium
Parodis, Ioannis - Cedersund, Gunnar - Eklund, Daniel - Kruse, Robert - Kurland, Lisa - Persson, Alexander -

11h30 Role of neutrophil proteinases in the local and systemic inflammation development
Kubyshkin, Anatolii - Fomochkina, Irina - Kovalenko, Evgeniia - Nomerovskaya, Aleksandra

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Special recognition: A tribute to Pr. Pierre Borgeat

23/07/2024 12:45-13:05 Room: 200A

12h45 A tribute to Pr. Pierre Borgeat

Plenary lecture: Into thin air: Hypoxia and inflammation

23/07/2024 13:05-14:00 Room: 200A

13h05 Into thin air: Hypoxia and inflammation

Symposium Canada-2: Steps along the pain pathway
Salvemini, Daniela - McDougall, Jason

23/07/2024 14:00-15:15 Room: 200A

14h00 Nociceptor desensitization as a first step to control inflammatory pain

14h15 Neuron-macrophage communication in the dorsal root ganglia modulates chronic pain

14h30 Discovery of novel spinal inflammatory pathways that contribute to pain

14h45 How microbial pathogens affect pain and itch

Symposium Australia: Targeting immunometabolism in inflammatory diseases
Sweet, Matthew - Hansbro, Phil

23/07/2024 14:00-15:15 Room: 301B

14h00 Metabolic features of mucosal tissues shaping inflammatory disease

14h15 Clonal haematopoiesis is influenced by metabolic disorders

14h30 An Innate Immune Signalling Axis That Controls Inflammatory Cytokine Production Via Regulated Immunometabolism
Sweet, Matthew - Das Gupta, Kaustav - Ramnath, Divya - von Pein, Jessica - Curson, James - Wang, Yizhuo - Abrol, Rishika - Gunther, Kimberley

14h45 Oxidized Cholesterols Drive Macrophage Recruitment and Inflammation in the Lung

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Inflammation and musculoskeletal disorders

Pelletier, Martin

23/07/2024 15:30-16:45 Room: 200A

15h30 Changes in Extracellular Matrix Remodeling by Lonomia Obliqua Caterpillar Proteins in an In Vitro Inflammatory Cell Model of Osteoarthritis
Alvarez-Flores, Miryam Paola - Gomes, Renata N. - Oliveira, Douglas S. - Melo, Amanda T. - de Melo, Thatiana C. - Buri, Marcus V. - DeOcesano-Pereira, Carlos - Goldfeder, Mauricio B. - Chudzinski-Tavassi, Ana Marisa

15h45 Therapeutic strategies to mitigate diastolic dysfunction in arthritis: targeting IL-6 and Galectin-3
Christoforou, Marilena - Chen, Jianmin - Cooper, Dianne - Perretti, Mauro

16h00 Lymphocyte, NK cell and mitochondrial gene dysregulation patterns separate patients with neuropsychiatric systemic lupus erythematosus into distinct subgroups with differential anticipated response to targeted therapies
Lindblom, Julius - Nikolopoulos, Dionysis - Toro-Domínguez, Daniel - Carnero-Montoro, Elena - Borghi, Maria Orietta

16h15 Microbiota is not essential for the development of popliteal lymphatic vessel hyperactivity in the TNFARE/+ arthritic mouse model
Neto de Jesus, Flavia - Defaye, Manon - Roizes, Simon - Liao, Shan - von der Weid, Pierre-Yves

16h30 Longitudinal Analysis of Key Blood Biomarkers in Early Arthritis Patients
Hasse, Stephan - Mortazavi, Helya - Boilard, Eric - Fortin, Paul R. - Julien, Anne-Sophie - Bourgoin, Sylvain G.

Specialized pro-resolving mediators (SPMs)

Chiang, Nan - Simard, Melissa

23/07/2024 15:30-16:45 Room: 301B

15h30 Potential of Protectin DX analogues as novel antidiabetic therapeutics
Desmarais, Frederik - Maltais, René - Sancéau, Jean-Yves - Marcotte, Bruno - Guèvremont, Geneviève - Trottier, Jocelyn - Mitchell, Patricia - Barbier, Olivier - Poirier, Donald - Marette, André

15h45 Pro-resolving lipid mediators prevent cancer cachexia
Panigrahy, Dipak - Haak, Victoria - Bayer, Rachel - Quinlivan, Katherine - Smith, Keira - Freedman, Steven - Yang, Haixia - Serhan, Charles

16h00 Deficiency in Platelet 12-Lipoxygenase exacerbates inflammation and Disease Severity During SARS-Cov-2 Infection
Andrade, Ana Cláudia - Lacasse, Emilie - Dubuc, Isabelle - Gudimard, Leslie - Puhm, Florian - Campolina-Silva, Gabriel - Queiroz-Junior, Celso - Allaeys, Isabelle - Prunier, Julien - Dumais, Élizabeth - Flamand, Nicolas - Droit,

16h15 Interferon-B evokes lipid class switching to regulate neutrophil functions and to drive the resolution of acute lung inflammation
Rizo-Tellez, Salma A. - Sekheri, Meriem - Othman, Amira - El Kebir, Driss - Filep, János G.

16h30 Stereocontrolled Total Syntheses of Resolvin-Epoxide Intermediates and their Transformation to Potent Pro-resolving Mediators by Human Leukocytes
Nshimiyimana, Robert - Simard, Melissa - Serhan, Charles

Phagocytes

Sirois, Martin

23/07/2024 15:30-16:45 Room: 301A

15h30 Maresin conjugates in tissue regeneration (MCTRs) improve post-influenza pneumococcal pneumonia by reprogramming of macrophages and modulation of inflammation
Padua Tavares, Luciana - Bruggemann, Thayse - Rezende, Rafael M. - G. Machado, Marina - Cagnina, Elaine - E. Shay, Ashley - C. Garcia, Cristiana - Nijmeh, Julie - Serhan, Charles - Teixeira, Mauro - Levy, Bruce D.

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15h45 Pharmacological Evidences That the Inhibitory Effects of Prostaglandin E2 Are Mediated by the EP2 and EP4 Receptors in Human Neutrophils
Lavoie, Jean-Philippe C. - Simard, Mélissa - Kalkan, Hilal - Rakotoarivelo, Volatiana - Huot, Sandrine - Di Marzo, Vincenzo - Côté, Andréanne - Pouliot, Marc - Flamand, Nicolas

16h00 Low-density neutrophils in adults with cystic fibrosis are associated with lung disease progression and display distinct antimicrobial capacity
Murru, Andréa - Vadeboncoeur, Nathalie - Boucher, Élise - Coderre, Lise - Labrecque, Marie-Michèle - Berthiaume, Yves - Bouvet, Guillaume - Adam, Damien - Brochiero, Emmanuelle - Lesage, Sylive - Flamand, Nicolas - Bilodeau,

16h15 Does Polymorphonuclear Leukocyte Activation Explains Acute Reduction in Cognitive Function in Human Subjects?
Kipen, Hoiward - Lu, Frederic - Gupta, Disha - Fiedler, Nancy - Satish, Usha - Black, Kathleen - De Resende, Adriana - Calderon, Leonardo - Guo, Changjiang - Gow, Andrew

16h30 Regulation of Macrophage Activation and Disease Pathogenesis following Ozone Exposure by Extracellular Vesicles and miRNA Cargo
Laskin, Debra - Sunil, Vasanthi - Vayas, Kinal - Abramova, Elena - Jin, Yang - Businaro, Rita - Laskin, Jeffrey

COVID-19
Grandvaux, Nathalie

23/07/2024 15:30-16:45 Room: 303B

15h30 Acute Antiviral and Pro-Resolving Interventions Prevent Neuropsychiatric Sequelae in an Experimental Model of Post-COVID Syndrome
Pimenta, Jordane - Beltrami, Vinícius - Oliveira, Bruna - Queiroz-Junior, Celso - Barsalini, Jessica - Teixeira, Danielle - Souza-Costa, Luiz Pedro - Lima, Anna Luiza - Machado, Caroline - Parreira, Bárbara - Santos, Felipe - Costa,

15h45 Inhibition of the C5a/C5aR1 Pathway: Controlling inflammation from Bench to Bedside
Guo, Renfeng - Habel, Maria - Xu, Zhongli - Liu, Rui - Burnette, Bruce - Chong, Camilla - Riedemann, Niels

16h00 Impact of a dual S1P1 S1P5 receptor ligand on COVID-19 immunopathology: exploratory analyses of the randomized, open-label, COZI pilot trial
Marsolais, David - Blais-Lecours, Pascale - Chateauvert, Nathalie - Rola, Philippe - Nguyen, Tuyen - Lellouche, François - Lesage, Sylvie - Courtemanche, Olivier

16h15 Role of IL-17RD in Antiviral Innate Immunity
El-Mortada, Firas - Girondelle, Charlotte - Dos Santos Pereira Andra, Ana Claudia - Dubuc, Isabelle - Lacasse, Émile - Flamand, Louis - Meloche, Sylvain - Servant, Marc

Gut
Philpott, Dana

23/07/2024 15:30-16:45 Room: 303A

15h30 Proteolytic Activity Correlates with Tissue Permeability and Symptom Status in Crohn's Disease
Hann, Amber - Szeto, Janet - Bording-Jorgensen, Michael - Wang, Xuanyu - Constante, Marco - Surette, Michael - Armstrong, David - Moayyedi, Paul - Galipeau, Heather - Verdu, Elena

15h45 Proteases-Activated Receptors play a key role in postoperative ileus
Gauthier, Romain - Thevenin, Julie - Planchamp, Thibault - Berthon, Teo - Rousset, Perrine - Vergnolle, Nathalie - Buscail, Etienne - Deraison, Céline

16h00 Translating Pharmacokinetic and Efficacy Outcomes of NLRX1 Agonist NX-13: Contrasting a Pig Model and a Human Phase 1b Clinical Trial In Ulcerative Colitis
Danese, Silvio - Verstockt, Bram - Dubinsky, Marla - Mosig, Rebecca - Yarur, Andres - Cataldi, Fabio - Siegmund, Britta

16h15 Visceral Pain in Inflammatory Bowel Disease: a role for Protease-Activated Receptor-1
Vergnolle, Nathalie - Rolland, Corinne - Rousset, Perinne - Buscail, Etienne - Buscail, Louis - Bournet, Barbara - Le Cosquer, Guillaume - Sablayrolles, Sylvie - Le Grand, Bruno - Deraison, Céline

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16h30 Intestinal elastolytic is involved in the control of visceral pain and hypersensitivity
Dumas, Alexia - Castro, Marta - Rousset, Perrine - Guiraud, Laura - Edir, Anissa - Sagnat, David - Cenac, Nicolas - Neunlist, Michel - Barbara, Giovanni - Rolland, Corinne - Motta, Jean-Paul - Deraison, Céline - Vergnolle, Nathalie

Plenary lecture: Microbiota and intestinal inflammation

24/07/2024 08:30-09:30 Room: 200A

8h30 Microbiota and intestinal inflammation

Symposium Canada-3: Microbiota control as a way out of intestinal inflammation: Highlight on young investigators

24/07/2024 09:30-11:00 Room: 200A

9h30 The emerging role of microbial proteolytic activity in inflammatory bowel disease

9h45 Thrombin tension at host-microbiota interface: A role in Crohn's disease?

10h00 Microbial antigen metabolism in adverse reactions to foods and intestinal inflammation

10h15 Neutrophils: From IBD to the gut microbiota

Symposium Canada-4: Brain-Gut actors of inflammation
Bercik, Premysl

24/07/2024 09:30-11:00 Room: 301B

9h30 Microbiota-gut-brain axis: From Irritable Bowel Syndrome (IBS) to Inflammatory Bowel Disease (IBD)

9h45 Neuroimmune mechanisms of visceral sensitization

10h00 Role of stress and the brain-gut axis in inflammatory gastrointestinal disorders

10h15 The role of the brain-gut axis in the development of chronic pain in IBD

INTERNATIONAL ASSOCIATION OF INFLAMMATION SOCIETIES

Abstract Book (Orals)

WCI2024.org

A Fault in the Pipeline: The Impact of Chronic Inflammation on Collecting Lymphatic Junction Integrity

Keith Keane[1], Matthew Stephens[1], and Pierre-Yves von der Weid[1]

[1] Inflammation Research Network, Snyder Institute of Chronic Diseases, Department of Physiology and Pharmacology, University of Calgary, Canada

Collecting lymphatic vessels (CLV) critically transport lymph and immune cells from tissue beds to lymph nodes and back to systemic circulation. Historically, and to perform this function, CLVs have been considered impermeable. However, recent studies have demonstrated increased permeability in situations such as inflammation. We challenged this concept further by assessing the junctional integrity of lymphatic endothelial cells (LECs) in mesenteric CLVs of the $TNF^{\Delta ARE/+}$mouse, a transgenic model of Crohn's disease which displays terminal ileal inflammation, dilation of mesenteric CLVs, clustering of immune cells at CLV valve sites leading to the formation of tertiary lymphoid organs, and leakage of inflammatory lymph from these vessels.

CLVs isolated from 12- and 28-week-old wild type (WT) and $TNF^{\Delta ARE/+}$ mice were fixed pressurised before being assessed for LEC changes to CD31 and VE-Cadherin distribution/expression by fluorescence confocal imaging. While there were no differences between WT and $TNF^{\Delta ARE/+}$ vessels at 12 weeks, there was a significant loss in junctional protein expression along the length of the 28-week $TNF^{\Delta ARE/+}$ vessels as well as a distinct loss in junctional protein organization at valve sites. These sites also demonstrated a loss of the CLV-identifying marker FOXC2.

To determine whether the overexpressed TNFα altered junctional protein expression and permeability, WT vessels were incubated with 100ng/ml of TNFα for 24 h and compared with untreated vessels. Only CD31 staining was significantly reduced in the post-valve regions of the vessels, which were also more permeable to luminally perfused dextrans (3-5 kDa and 70 kDa) than control vessels.

In conclusion, chronic inflammation in $TNF^{\Delta ARE/+}$ mice increases the permeability of mesenteric CLVs by decreasing junctional protein expression and altering their cell surface distribution. While TNFα contributes to this loss by decreasing CD31 expression in the post-valve region and increasing vessel permeability, the loss of junctional organization may require a longer exposure to TNFα and other inflammatory mediators. We are currently examining whether differences in shear stress experienced in specific regions of the CLVs alter their sensitivity to TNFα and the expression of their junctional proteins.

Acute Antiviral and Pro-Resolving Interventions Prevent Neuropsychiatric Sequelae in an Experimental Model of Post-COVID Syndrome

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The global impact of the COVID-19 pandemic is now compounded by a new challenge, Post-COVID Syndrome (PCS), affecting over 65 million individuals. This study aimed to: (i) comprehensively characterize acute effects of MHV-A59 betacoronavirus inoculation in mice; (ii) investigate possible PCS manifestations: (iii) assess gender-specific differences, and (iv) to explore the impact of acute antiviral (Remdesivir) or a pro-resolving (the melanocortin agonist, AP1189) interventions on the establishment and severity of PCS. Male and female C57Bl/6 mice were inoculated with 3×10^4 PFU of MHV-A59. Mice exhibited clinical (body weight loss) and haematological (altered neutrophil/lymphocyte ratio) from the 2^{nd} to 5^{th} days post-infection (dpi). Marked lung lesions were characterized by hyperplasia of the alveolar walls, infiltration of polymorphonuclear leukocytes (PMN) and mononuclear leukocytes, hemorrhage, increased concentrations of CCL2, CCL3, CCL5, and CXCL1 chemokines, as well as high viral titers until the 5^{th} dpi. While these lung inflammatory signs resolved, other manifestations were observed up to the 60^{th} dpi, including mild brain lesions with gliosis and hyperemic blood vessels, reduced BDNF levels and augmented $CD4^+$ T cells producing IFN-γ in the brain, along with neuromuscular dysfunctions, anhedonic-like behavior, deficits in spatial working memory, and short-term aversive memory. These musculoskeletal and neuropsychiatric complications were exclusive to female mice and were prevented after ovariectomy. Similar results were observed in SARS-CoV-2 infected mice. Finally, early antiviral or pro-resolving interventions (from day 2 to 5 pi) completely prevented neuropsychiatric sequelae induced by MHV-A59 infection .This study unveils a novel sex-dependent PCS model with a focus on neuropsychiatric and musculoskeletal disorders, offering insights into acute therapeutic interventions' impact on betacoronavirus-induced long-term sequelae.

Ameliorating Cancer Cachexia by Inhibition of Soluble Epoxide Hydrolase

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Background: Cancer cachexia is a devastating syndrome characterized by progressive muscle wasting and hyperinflammation. While the underlying mechanisms remain poorly characterized, there are no effective treatments for cancer cachexia. Cancer cachexia is driven by systemic inflammation and pro-inflammatory cytokines. Arachidonic acid-derived epoxyeicosatrienoic acids (EETs) are anti-inflammatory lipid mediators which stimulating inflammation resolution. EETs are rapidly metabolized by the enzyme soluble epoxide hydrolase (sEH). Thus, we hypothesized that pharmacological inhibition of the sEH may prevent cancer cachexia via the resolution of inflammation. **Methods:** We investigated murine cancer cachexia models using genetically engineered pancreatic (KPC) and prostate (TRAMP C1) tumor cell lines, and genetically engineered mice (transgenic adenocarcinoma of the mouse prostate-TRAMP). Cachectic mice were treated with human sEH inhibitor EC5026. **Results:** An increase in sEH expression was observed in the spleen, liver, heart, gastrocnemius (GA), and tibialis anterior (TA) muscles compared to healthy controls by IHC and qPCR (n = 5/group). KPC cells injected intraperitoneally induced a reduction in the weights of the spleen, liver, heart, brain, GA, and TA compared to healthy controls (n = 15/group). KPC mice treated with EC5026 showed increased and comparable organ weights to healthy controls. In addition, treatment with EC5026 significantly improved survival rates in both KPC and TRAMP models. A sustained survival of over 250 days was observed post-injection in the KPC model (n=15 mice/group). In the TRAMP model, 5/5 of mice treated with EC5026 survived 230 days post-treatment compared to no survival of vehicle-treated mice (n=5). Mice treated with EC5026 showed a reduction in sEH expression in all tissues. Additionally, qPCR analysis of gastrocnemius tissue indicated that sEH inhibition attenuated pro-inflammatory markers and eicosanoid enzymes, evidenced by reduced expression of IL-6, NF-kB, LTB4R, Cox-2, Alox5, Alox12, Cyp2j5, and Cyp2c65 comparatively (n=5) and increased pro-resolving receptors (e.g. RvD2/GPR18). Immunoprofiling by flow cytometry (n=15/group) revealed a significant increase in macrophages, CD8+ T cells, and NK cells, and a decrease in CD4+ T cells in EC5026-treated KPC mice across various cachexia tissues. KPC mice treated with EC5026 expressed similar percentages of immune cell populations compared to healthy controls in each tissue type. **Conclusions:** Thus, sEH inhibition may be a novel host-directed therapeutic approach to cancer cachexia by targeting the host immune response via stimulation of resolution of inflammation without toxicity or immunosuppression.

An innate immune signalling axis that controls inflammatory cytokine production via regulated immunometabolism

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Innate immune cells sense danger in different contexts, allowing inflammatory responses to be tailored to the level of threat encountered. Here we describe a metabolic switch that triages threats, either switching on or dampening macrophage inflammatory responses, depending on the type of danger detected[1]. We also reveal novel immunoregulatory properties of the macrophage metabolite ribulose-5-phosphate (RL5P)[1]. We show that histone deacetylase 7 (HDAC7) is a cytoplasmic lysine deacetylase in macrophages. When danger signals such as soluble lipopolysaccharide (LPS) indicating far-away or distal danger are detected by macrophages, HDAC7 deacetylates the glycolytic enzyme PKM2 for HIF-1α activation and proinflammatory IL-1β production. In contrast, HDAC7 triggers the pentose phosphate pathway (PPP), NADPH production, and phagocyte oxidase-mediated reactive oxygen species generation for bacterial killing when nearby threats, such as bacteria, are encountered. PPP activation by HDAC7 also suppresses IL-1β and other inflammatory cytokines, allowing cells to focus resources on bacterial killing. *In vivo* targeting of HDAC7 compromises antibacterial defense and exacerbates inflammation, highlighting the pivotal role of this pathway during infection. Mechanistically, HDAC7 activates the PPP enzyme 6-phosphogluconate dehydrogenase (6PGD), with the enzymatic product of 6PGD, RL5P, suppressing inflammatory responses in human and mouse macrophages. RL5P also exerts direct antibacterial effects on *E. coli* exposed to oxidative stress, suggesting this metabolite may also contribute to bacterial killing in the macrophage phagosome. In summary, HDAC7 engages the glycolysis-PKM2-HIF-1α axis to drive inflammation when signs of far-away danger are detected, but triggers the PPP-6PGD-RL5P pathway for direct antibacterial defense over proinflammatory cytokine production when nearby danger is encountered. Thus, the HDAC7 immunometabolic switch selects for inflammatory versus antimicrobial responses in macrophages and the HDAC7-6PGD-RL5P axis may be amenable for targeting in inflammation and/or infection.

References

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Changes in Extracellular Matrix Remodeling by *Lonomia Obliqua* Caterpillar Proteins in an In Vitro Inflammatory Cell Model of Osteoarthritis

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Chondrocytes are responsible for producing and maintaining the extracellular matrix of cartilage, whose proteins undergo changes under inflammatory conditions observed in join diseases. Several reports have shown that animal venoms display immunomodulatory and anti-inflammatory effects in arthritis models. Animal venoms are characterized by the unique biochemical diversity of their toxins and the repertoire of cellular receptors and signaling pathways they target to fulfill their role in poisoning. This opens new perspectives for the discovery of of new targets for therapeutic intervention in inflammatory joint diseases. Lonomia obliqua caterpillar venom is well-known to cause a hemorrhagic syndrome and systemic proinflammatory response in patients that become in contact with its urticating's bristles. Lopap and Losac are both cytoprotective proteins derived from L. obliqua bristles extract (LOCBE). In this context, the aim of this study was to determine if LOCBE and recombinant Lopap and Losac are able to modulate ECM lost under inflammatory conditions induced by interleukin 1β in chondrocytes. Both molecules were able to reduce the loss of ECM proteins involved in tissue remodeling (as collagen) and to preserve substrate attachment and ECM integrity. LOCBE was able to increase the mitochondrial activity with no effect on NF-kB pathway. However, LOCBE increased the expression of nuclear MMP-1, a well-known collagenase with an important role in apoptosis and ECM remodeling. Analysis of chondrocytes with Lopap and Losac revealed that Lopap induced the higher expression of nuclear MMP-1 and increased mitochondrial activity. Thus, MMP-1 could be an important target being modulated by LOCBE and the recombinant proteins and related to their cytoprotective effects widely studied. We demonstrated that LOCBE and recombinant proteins Lopap and Losac are important sources for the study of cytoprotection, apoptosis and the mechanism of nuclear MMP-1, which is no longer a well-understood process. Further studies must be performed to elucidate the biological relevance of this effect in the envenoming, and if there are, other pathways activated without the involvement of the NF-kB pathway.

Deficiency in Platelet 12-Lipoxygenase exacerbates inflammation and Disease Severity During SARS-Cov-2 Infection

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Platelets, traditionally known for maintaining blood balance, are now recognized as crucial cells in antimicrobial defense. Upon SARS-CoV-2 infection, platelets become hyperactivated, releasing various molecules such as cytokines, granule contents, and bioactive lipids. The key biolipids produced in platelets are regulated by enzymes: group IV cytosolic phospholipase A2 (cPLA2α) responsible for the hydrolysis of precursor lipids from membrane phospholipids; 12-lipoxygenase (12-LOX), producing 12-hydroxyeicosatetraenoic acid (12-HETE); and cyclooxygenase-1 (COX-1), implicated in prostaglandins (PG) and thromboxane (TX) production. While PGE2 and TXB2 were previously associated with lung inflammation in severe COVID-19, the role of platelet 12-LOX in SARS-CoV-2 infection remains unclear. Using *Pla2g4a* and *Alox12* knockout mice, we found increased lung inflammation in *Pla2g4a* knockout mice post-infection. Conversely, *Alox12*-deficient mice exhibited not only higher lung inflammation characterized by increased leukocyte infiltrates and cytokine productions but also premature mortality and distinct lung transcriptomic changes, including alterations in NLRP1 inflammasome-related gene expression. Additionally, lipidomic analysis in *Alox12* knockout mice revealed significant changes, including reduced levels of the 12-LOX product 12-HETrE and Maresin-2, known for their anti-inflammatory and pro-resolving effects, inversely correlating with disease severity. This study highlights the complex interplay between 12-LOX-related lipid metabolism, and inflammatory responses during SARS-CoV-2 infection. The findings provide valuable insights into potential therapeutic targets aimed at mitigating severe outcomes, emphasizing the pivotal role of platelet enzymes in the host response to viral infections.

Discoidin domain receptor 1 (DDR1) contributes to the development of arthritis by promoting the migration of pathogenic Th17 cells through collagen.

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Th17 cells play an important role in autoimmune diseases, but the mechanisms regulating their migration into inflammatory tissues are not fully understood. We have previously reported that the discoidin domain receptor 1 (DDR1) enhances the migration of human polarized Th17 cells into collagen gels. In this study, we examined the role of DDR1 in arthritis. We found that human pathogenic Th17 cells (pTh17) also express and use DDR1 to migrate into collagen gels. The DDR1 kinase inhibitor (7rh) reduced DDR1 activation and the migration pTh17 cells into collagen gels. The effect of 7rh was confirmed by expressing the DDR1 dominant-negative form in human Th17 cells. We then examined the role of DDR1 in the mouse model of collagen-induced arthritis (CIA). Treatment of CIA mice with 7rh reduced the severity of arthritis, synovial inflammation and cartilage destruction. Blocking DDR1 also reduced Th17 cell infiltration into the joints, reduced proinflammatory cytokines and increased IL-10 cytokine levels suggesting that DDR1 is an important pathogenic pathway in arthritis.
Our results indicate that DDR1 inhibition could reduce inflammation and tissue damage in collagen-rich tissues, like arthritic joints, and may therefore represent a new therapeutic target in autoimmune arthritis.

Does Polymorphonuclear Leukocyte Activation Explains Acute Reduction in Cognitive Function in Human Subjects?

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Introduction and Rationale: Acute human exposures to common indoor concentrations of 1,000-2,500 ppm of CO_2 cause significant cognitive deficits in measures of executive function. Rodent inhalation studies at these concentrations leads to PMN activation, oxidative burst, and systemic vascular inflammation, and vascular leak in brain, potentially providing a mechanism for the observed declines in executive function. We sought to test if mechanistic findings in rodents are observed in human subjects.

Methods: Twelve college students completed standardized tests of executive function (Strategic Management System, SMS) for 2 h while breathing in a chamber , one week apart, either filtered ambient air (CO_2=600 ppm) or air with CO_2 at 2,500 ppm, in a blinded, randomized within subject cross-over design. Four hours after exposure, isolated PMNs were examined for markers of activation.

Results: CO_2 exposure impaired SMS performance, and increased baseline O_2 consumption from 13 ± 2.9 to 21 ± 3.3 pmole/min. For oxidative burst, neutrophils were treated with PMA, and basal O_2 consumption was measured. There was a significant increase in O_2 consumption; however, relative to air, CO_2 exposure delayed the time to peak from 56 ± 3.5 to 98 ± 9.2 minutes. Additionally, CO_2 reduced the total oxidative burst as determined by the area under the curve (11 ± 0.9 vs. 8 ± 1.3 nmoles). All changes were statistically significant.

Discussion: CO_2 exposure increased non-mitochondrial O_2 consumption without external stimulation. This was observed in all subjects and is indicative of increased NADPH oxidase function. Most importantly, CO_2 abrogated the PMA-mediated oxidative burst presumably as the cells were already stimulated. These data support an inflammatory mechanism for the loss of executive function following CO_2

Eosinophil phenotypes are functionally regulated by resolvin D2 during allergic lung inflammation

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Eosinophils (Eos) reside in multiple organs during homeostasis and respond rapidly to an inflammatory challenge. Although Eos share chemical staining properties, they also demonstrate phenotypic and functional plasticity that is not fully understood. Here, we used a murine model of allergic lung inflammation to characterize Eos subsets and determine their spatiotemporal and functional regulation during inflammation and its resolution in response to resolvin D2 (RvD2), a potent specialized pro-resolving mediator. Two Eos subsets were identified by CD101 expression: $CD101^{low}$ Eos, that were found in naïve lungs and during inflammation, and $CD101^{high}$ Eos that were present only during inflammation. $CD101^{low}$ and $CD101^{high}$ Eos had distinct anatomic localization as the first being found predominantly in the lung vascular niche and the second mainly located in bronchoalveolar space. $CD101^{low}$ Eos responded to allergen challenge by increasing their activation and migrating into the lung interstitium and bronchoalveolar space to then become $CD101^{high}$ Eos. This process was governed in part by IL-5. RvD2 reduced total number of Eos, specially $CD101^{high}$ Eos in bronchoalveolar lavage and changed their activation phenotype by at least two distinct mechanisms: decreasing IL-5-dependent recruitment of $CD101^{low}$ Eos and decreasing conversion of $CD101^{low}$ Eos to $CD101^{high}$ Eos. Collectively, these findings indicate that Eos are a heterogeneous pool of cells with distinct activation states and spatiotemporal regulation during resolution of inflammation and that RvD2 is a potent pro-resolving mediator for Eos recruitment and activation.

EP 80317, a CD36 ligand, attenuates remote lung injury after transient hind limb ischaemia in mice

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The revascularisation of ischemic skeletal muscle is associated with remote organ injury including lungs. Mobilisation of activated polymorphonuclear neutrophils and monocyte to the lungs after the reperfusion of ischemic lower limb results mainly from the release of inflammatory mediators from the ischemic tissue and leukocyte trapping into the remote organ. The CD36 scavenger receptor (SR-B2) is widely expressed in many immune and non immune cells. In this study, we investigated the effect of a CD36 receptor ligand, EP 80317 (Haic-D-Trp(2-Me)-D-Lys-Trp-D-Phe-Lys-NH2), in lung inflammation after hind limb ischemia-reperfusion in wild-type C57BL/6J mice. All the experiment protocols have been approved by the Institutional Animal Ethics Committee under the guidelines of the Canadian Council for Animal Care. Male C57BL/6J mice ($CD36^{+/+}$ and $CD36^{-/-}$) were pretreated daily for 14 days with 300 μg/kg of EP 80317 or 0.9% vehicle subcutaneously. Mice, under isoflurane anaesthesia, were subjected to 30 minute occlusion of the right hind limb blood flow by the application of a rubber band upstream of the femoral muscle, followed by 3 hours of reperfusion. Mice were euthanized by an overdose of isoflurane and exsanguination, and tissues were collected. The results show a marked reduction of leukocytes in lung homogenates as assessed by the myeloperoxidase assay, by 39% in EP 80317-treated mice compared to vehicle treated mice ($p<0.001$). In contrast, no change was observed in $CD36^{-/-}$ mice. Interleukin-1beta levels were reduced by 29% ($p<0.05$) in the lung homogenate of mice treated with EP80317. Morover, leukotriene B4 (LTB_4) and prostaglandin E_2 (PGE_2) levels were reduced by 22% ($p<0.05$) and 53% ($p<0.05$), respectively in lung homogenates whereas no change was observed in Cd36-deficient mice. A reduction in malonyldialdehyde concentrations of 49% ($p < 0.05$) was observed, but these levels remained unchanged in CD36-deficient mice. These results show, for the first time, that targeting the CD36 receptor reduces lung inflammation by attenuating the generation of the lipid mediators LTB_4 and PGE_2, in addition to reducing cytokine and systemic indices of oxidative stress. These results suggest that the CD36 receptor could be a target for the treatment of remote pulmonary inflammation.

Fpr1/Fpr2-Based Resolution Pharmacology Attenuates Cardiomyopathy in Inflammatory Arthritis

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Rheumatoid arthritis (RA) patients face a two-fold increased risk of cardiovascular diseases, including heart failure [*Lancet 2022; 400: 733-43*]. Notably, they are particularly prone to heart failure with preserved ejection fraction, marked by diastolic dysfunction. The causes of diastolic dysfunction in RA remain unknown, while existing medications provide limited cardioprotection. Addressing this clinical gap, we characterised the first mouse model mirroring left ventricular diastolic dysfunction in RA patients, the K/BxN F1 mice [*PNAS 2021; 118(38):e2020385118*], which present diastolic dysfunction ~4 weeks after development of arthritis. Our focus centered on the annexin A1 (AnxA1)/formyl-peptide receptor type 2 (FPR2) pro-resolving pathway. Echocardiography-monitored cardiac diastolic dysfunction correlated with immune cell infiltration and fibroblast proliferation. Prophylactic or therapeutic treatment with 1 µg *s.c.* hrAnxA1 daily halted or reversed cardiomyopathy, respectively. These effects correlated with decreased fibroblast numbers and activation, and macrophage skewing toward an M2-like polarisation.

Given that both Fpr1 and Fpr2 mRNA expression are elevated in K/BxN F1 mouse hearts, we tested here relevant agonists, addressing also if selective Fpr2 agonism adds value as compared to a dual Fpr1/Fpr2 agonist. To this end, we tested two small molecules: BMS986235 (Fpr2 selective) and Compound43 (dual Fpr1/Fpr2 agonist) at 3 mg/kg and 10 mg/kg per os, daily from week 4. Both compounds prevented cardiac dysfunction (monitored from week 4 to 8) but targeted distinct cellular types (quantified at end of the experiment). BMS986235 reduced activated T cells, M1-like macrophage proportion, and fibroblast numbers. Conversely, Compound43 reduced galectin-3 expression in fibroblasts mitigating pro-fibrotic activity, and decreased pro-inflammatory monocyte and macrophage infiltration. Neither treatment impacted on the degree of arthritis.

In summary, these findings suggest a potential for FPR-based pharmacological interventions in treating inflammatory arthritis-associated cardiomyopathy.

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Identification of a novel macrophage subset involved in pulmonary fibrosis by intravital imaging techniques

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Following tissue damage by internal and external factors, tissue repair occurs through the dynamic interaction of a wide variety of cells; however, fibrosis can also occur as a result. To understand the complex cellular and molecular basis of fibrosis, it is necessary to analyze the cellular processes involved *in vivo*. This study aimed to develop an imaging system for visualizing the dynamics of immune cells in the lung using intravital two-photon microscopy and to elucidate the mechanisms involved in the pathogenesis of pulmonary fibrosis, a disorder for which there are few available treatments. By means of the intravital lung imaging system, we visualized cellular morphology and motility in the lung of bleomycin-induced pulmonary fibrosis *in vivo*. We identified a novel macrophage subset, which was not observed under homeostatic conditions, appearing adjacent to pulmonary blood vessels, followed by the development of fibrosis. To examine whether these macrophages are involved in the pathogenesis of fibrosis, we sorted these cells from fibrotic lungs and transferred them into the trachea of healthy recipient mice. Intravital imaging showed that the amount of collagen fibers significantly increased in the lung after adoptive transfer of cells, suggesting that they have the capacity to induce fibrosis. Furthermore, RNA-sequence analysis demonstrated that these macrophages expressed higher levels of several fibrotic markers, compared to other types of macrophages. We also found that myeloid cell-specific deletion of fibrotic markers alleviated pulmonary fibrosis *in vivo*. In conclusion, we established an intravital lung imaging system for visualizing fibrosis and identified a novel macrophage population in the lungs at the onset of fibrosis. This approach will yield compelling insights into the molecular mechanisms underlying fibrosis, which could also serve as the basis for developing novel anti-fibrotic therapies.

Impact of a dual S1P1 S1P5 receptor ligand on COVID-19 immunopathology: exploratory analyses of the randomized, open-label, COZI pilot trial

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Sphingosine-1-phosphate (S1P) receptor ligands reduce lung damage and endothelial activation in models of virus-induced pneumonia. We documented the feasibility of administering an S1P receptor ligand to hospitalized COVID-19 patients (NCT04405102; *Chest, Oct 28th 2023*). Clinical, biochemical, immunological data were obtained longitudinally and exploratory analyses relating to the effects of an S1P receptor ligand on COVID-19 pathognomonic features will be presented.

Twenty-three patients were randomized to standard of care (SOC), and 20 were randomized to receive standard of care plus ozanimod (OZA; oral, once daily for a maximum of 14 days). The OZA group showed nonsignificant reductions of median time to clinical improvement (4 [range, 3-7] vs 7 [range, 3-11] days; p =0.12) for SOC. Ozanimod appeared to prevent the increase of circulating neutrophils and monocytes, the latter likely involving a decreased proportion of classical monocytes. Interestingly C-reactive protein circulating levels were lower for the OZA group on study day 7 (p=0.06) and similar trends were observed for IL-8 and CCL2 on study day 3. We also found that ozanimod dampened the circulating levels of SARS-CoV-2 S1 protein-specific IgGs (p=0.06) on study day 90, which was overcome by the administration of a vaccine shot.

This is the very first study investigating putative leads on the mechanisms of action of S1P receptor ligands for patients with acute pneumonia. We found that ozanimod likely interferes with the kinetics of neutrophil and classical monocyte circulation, may affect specific cytokines linked with S1P signalling and ARDS, and that it may interfere with the primary humoral response to infection, while this can be mitigated by vaccination. Our findings recapitulate some of the beneficial mechanisms of S1P receptor ligands previously seen in animal models of virus-induced ARDS. Our work may pave the way for the safe off label use of S1P receptor ligands in patients with severe pneumonia.

Title: Inhibition of the C5a/C5aR1 Pathway: Controlling inflammation from Bench to Bedside

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C5a, an end-product of complement activation, is one of the most potent inflammatory peptides, playing a crucial role in the pathogenesis of numerous inflammatory disorders. Vilobelimab, a first-in-class anti-C5a monoclonal antibody, recently received emergency use authorization (EUA) from FDA for treating critically ill adult COVID-19 patients within 48 hours of receiving invasive mechanical ventilation or extracorporeal membrane oxygenation. FDA granted the EUA based on Phase III clinical trial results (PANAMO trial, N=369). The trial demonstrated the life-saving potential of vilobelimab with a significant relative reduction in 28-day all-cause mortality by 23.9% compared to the placebo ($P<0.05$; non-stratified Cox regression). Furthermore, a prior preclinical study revealed that vilobelimab substantially improved systemic inflammation and acute lung injury in a monkey model infected with the influenza H7N9 virus. This improvement was associated with decreases in lung infiltrates of neutrophils and macrophages. In preclinical settings, blocking the C5a/C5aR1 axis was beneficial in controlling inflammation in rodent models of bacterial and malaria sepsis. The robust anti-inflammatory effect resulting from inhibiting the C5a/C5aR1 axis is primarily attributed to attenuation of the “cytokine storm” and neutrophil activation, thereby preventing tissue damage caused by the release of toxic components such as enzymes, oxidants, and neutrophil extracellular traps (NETs). Importantly, blocking the C5a/C5aR1 pathway requires a target-specific approach because upstream blockade of C5 has proven inefficient in controlling C5a generation in patients suffering from strong inflammatory conditions such as COVID-19. This inefficiency is attributed to the fact that C5a can be generated by direct enzymatic cleavage of C5 independent of the conventional complement pathways (classical, lectin, and alternative) and not inhibited by known upstream complement inhibitors such as eculizumab. Target-specific inhibition of the C5a/C5aR1 axis emerges as a powerful means to control inflammation, warranting further exploration in various other inflammatory diseases.

Interferon-β evokes lipid class switching to regulate neutrophil functions and to drive the resolution of acute lung inflammation.

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Dysregulated neutrophil function underlies acute respiratory distress syndrome (ARDS) that is associated with high mortality. We reported that bacterial DNA (CpG DNA) through TLR9 activation impairs bacterial clearance by neutrophils and identified IFN-β as a macrophage-derived cytokine that facilitate bacterial clearance and promotes the resolution of ARDS. However, little is known about the underlying mechanisms; albeit these would be essential for implementing precision treatment with IFN-β. Since the resolution of inflammation is skewed toward a pro-resolving lipid profile, we investigated whether IFN-β can exert its beneficial actions through modulating pro-resolving lipid mediator-based resolution mechanisms. Culture of human neutrophils with IFN-β, 15-epi-LXA_4 or RvD1 countered the survival cues from CpG DNA through reducing Mcl-1 expression. CpG DNA stimulated production of leukotriene B_4 (LTB_4), but not 15-epi-lipoxin A_4 (15-epi-LXA_4) and resolvin D1 (RvD1). IFN-β reduced LTB_4 release without evoking detectable increases in 15-epi-LXA_4 and RvD1 production. Unlike 15-epi-LXA_4 and RvD1, IFN-β did not restore CpG DNA-impaired phagocytosis and bacterial clearance. In a mouse model of ARDS evoked by CpG DNA and intratracheal instillation of *E. coli*, treatment with IFN-β at the peak of inflammation, restored impaired neutrophil phagocytosis, accelerated bacterial clearance, enhanced neutrophil apoptosis and efferocytosis, resulting in accelerated resolution of airway inflammation. CpG DNA or neutralizing endogenous IFN-β markedly enhanced bronchoalveolar lavage fluid levels of LTB_4, while reduced 15-epi-LXA_4 and RvD1 levels. Conversely, treatment with IFN-β markedly enhanced lavage fluid levels of 15-epi-LXA_4 and RvD1 and reduced LTB_4. Furthermore, selective blockade of the receptor ALX/FPR2 (which binds both 15-epi-LXA_4 and RvD1) with WRW4 partially blocked IFN-β-mediated resolution, leading to impaired bacterial clearance and persisting lung injury. Our results identify an intricate interplay between IFN-β and pro-resolving lipid mediators that signal through ALX/FPR2 to restore impaired neutrophil function and to facilitate the timely resolution of neutrophil driven ARDS.

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Intestinal elastolytic is involved in the control of visceral pain and hypersensitivity

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Mucosal proteolytic homeostasis plays a crucial role in the pathophysiology of functional GI disorders, including Irritable Bowel Syndrome (IBS), one of whose main symptom is abdominal pain and visceral hypersensitivity. Although the exacerbation of wide-spectrum proteolytic activity has been reported in these patients, the exact mechanism to explain how the proteolytic balance controls visceral pain, as well as the type of proteases involved remain to be elucidated. We focused our attention on elastolytic activity, which is known to be exacerbated in high-grade inflammatory diseases, and investigated whether such activity could also be implicated in low-grade inflammatory conditions such as IBS, contributing there to pain signals. To address this question, we used tissue biopsies from IBS patients, several animal models of visceral pain, and calcium signalling in dorsal root ganglia neuron cultures. Intestinal elastolytic activity was increased in tissues from IBS patients and strongly associated with epithelium. Elastolytic activity was also increased in visceral hypersensitivity or pain models induced respectively by stress sessions or mustard oil i.c. administration. Active elastolytic enzymes induced dose-dependent calcium signals in primary afferent neuron cultures and when administered i.c., could cause visceral hypersensitivity in response to colorectal distension. Because stress contributes to the development of IBS, and because we observed that strong elastolytic activity was associated with the epithelium from IBS patients, we investigated the regulation of elastolytic activity by human epithelium. The expression of Elafin, an endogenous inhibitor of elastolytic enzymes, was significantly decreased after exposure of colon organoid cultures to a cocktail of stress hormones. Finally, inhibition of colonic elastolytic activity by Elafin delivery protected mice from visceral hypersensitivity and pain symptoms. Altogether, these data demonstrated that elastolytic activity is dysregulated in IBS and is associated with activation of sensory neurons and pain signals. The nature and source of elastases still has to be identified, but this study highlights elastolytic activity as a potential new target in the management of low-grade inflammation associated visceral pain.

Investigating the role of endocannabinoid and immune system in the small intestine of severe obese subjects with type-2 diabetes

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Obesity is currently considered as a multifactorial chronic disease and is accompanied by health complications including type-2 diabetes (T2D). Growing evidence has shown that low-grade inflammation associated with obesity and subsequent loss of gut barrier function contributes to insulin resistance. The endocannabinoids (eCBs), 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA, anandamide), and their main targets, the cannabinoid CB_1 and CB_2 receptors, are all expressed in the intestine. CB_1 is present in metabolic tissues and regulates their function, while CB_2 is predominant in immune cells where it modulates inflammation. Deciphering gut eCB signaling, therefore, encompasses both energy metabolism and chronic low-grade inflammation associated with obesity.

We aimed to characterize the inflammatory status of the gut in response to long-term adaptation to obesity and T2D as well as to define how the human eCB system acts at the interface between energy metabolism and immunity.

We obtained human ileal samples from bariatric surgery in subject with and without T2D. We showed that intestinal and circulating levels of eCBs and eCB-like compounds are higher in subjects with T2D. Moreover, expression analysis of 200 genes involved in inflammatory processes revealed that the expression of *TLR4* and of genes coding for pro-inflammatory cytokines (i.e. *IL1β*, *IL6* and *IL15*) was lower in subjects with T2D. This apparent reduced local inflammatory status in the gut is opposed to the presence of a low-grade systemic inflammatory status, which has been previously reported in subjects with T2D, and this might be due in part to the fact that the T2D subjects were under treatment.

Our study highlights gut specific inflammation mechanisms possibly linked to the eCB system. Understanding this process could lead to the identification of new targets for the treatment of obesity and related metabolic disorders.

WCI 2024 abstract
Longitudinal Analysis of Key Blood Biomarkers in Early Arthritis Patients

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In rheumatoid arthritis (RA), various molecular factors contribute to disease progression. Platelet-derived extracellular vesicles (PEVs) induce inflammation in synovial fibroblasts, while ferritin serves as a disease activation biomarker. The autotaxin (ATX) pathway exacerbates inflammation, particularly bone erosions. Phosphatidylserine (PS), mediated by phosphatidylserine-specific phospholipase A1A (PLA1A), perpetuates inflammatory cascades. However, the existing literature lacks comprehensive information, with only one study addressing the proteome composition of red blood cell (RBC)-derived vesicles (REVs). We conducted a two-year assessment on 79 early arthritis (EA) patients and 30 age-sex-matched healthy controls. Blood parameters were analyzed via ELISA and FACS, including normality tests, non-parametric tests, correlation analyses, Principal Component Analysis, and Partial Least Squares. EA patients, with a mean baseline age of 59.14±12.41 years, showed significantly elevated PAC+ platelets, CD62+ platelets, PAC+CD62P+ platelets, ferritin, PS+ PEVs, PS+ RBCs, PS+ REVs, and PLA1A levels compared to controls. Correlation analysis revealed significant associations between ATX and ferritin, PAC+ platelets and ferritin, PAC+ platelets and PS+ RBCs, CD62P+ and ferritin, CD62P+ platelets and PS+ REVs, PAC+CD62P+ platelets and ferritin, and PAC+CD62P+ platelets andPS+ RBCs. Over two years, EA patients exhibited increased ATX, PLA1A, and PS+ PEV levels, whereas ferritin and PS+ REVs decreased. PAC+ platelets, CD62+ platelets, PAC+CD62P+ platelets, and PS+ RBCs exhibited fluctuations over the study period in EA patients. A significant association was found between PS+ PEVs at two years and new plaque formation. Notably, treatment did not impact platelet activation markers and EV levels. This study reveals significant fluctuations in blood biomarkers associated with EA. Elevated levels of platelet activation markers, ferritin, and PLA1A in EA patients underline their association with the disease. Over the study period, dynamic changes were observed, including increases in ATX, PLA1A and PS+PEVs, alongside decreases in ferritin and PS+ REVs, suggesting their potential as disease activity indicators. The association between PS+ PEVs and increased plaque formation risk accentuates their clinical relevance.

Low density neutrophils and neutrophil extracellular traps (NETs) are new inflammatory players in heart failure

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Heart failure with reduced (HFrEF) or preserved ejection fraction (HFpEF) is characterized by low-grade chronic inflammation. Circulating neutrophils regroup two subtypes termed high- and low-density neutrophils (HDNs and LDNs). LDNs represent less than 2% of total neutrophil under physiological conditions, but their count increase in multiple pathologies, releasing more inflammatory cytokines and neutrophil extracellular traps (NETs). We wanted to assess the differential count and role of HDNs, LDNs and NETs-related activities in HF patients. HDNs and LDNs were isolated from human blood by density gradient and purified by FACS and their counts obtained by flow cytometry. NETs formation (NETosis) was quantified by confocal microscopy. Circulating inflammatory and NETosis biomarkers were measured by ELISA. Neutrophil adhesion onto human extracellular matrix (hECM) was assessed by optical microscopy. A total of 140 individuals were enrolled, including 33 healthy volunteers (HV), 41 HFrEF (19 stable patients and 22 presenting acute decompensated HF; ADHF) and 66 HFpEF patients (36 stable patients and 30 presenting HF decompensation). HDNs and LDNs counts were significantly increased up to 39% and 2740% respectively in HF patients compared to HV. In HF patients, the correlations between LDNs counts and circulating inflammatory (CRP, IL-6 and -8), Troponin T, NT-proBNP and NETosis components were all significant. In vitro, LDNs expressed more H3Cit and NETs and were more pro-adhesive, with ADHFpEF patients presenting the highest pro-inflammatory profile. In conclusion, HFpEF patients present higher levels of circulating LDNs and NETs related activities, which are the highest in the context of acute HF decompensation.

Low-density neutrophils in adults with cystic fibrosis are associated with lung disease progression and display distinct antimicrobial capacity

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Neutrophils are massively recruited to combat infections in the lungs of people with cystic fibrosis (PwCF), yet they fail to eradicate pathogens and thus contribute to chronic lung inflammation and loss of lung function. In addition to the circulating normal-dense neutrophils (NDNs), high numbers of low-density neutrophil subtypes (LDNs) have been reported in chronic inflammatory diseases such as lupus [1]. LDNs have immunophenotype and function distinct from those of NDNs and are associated with disease severity, activity, and certain comorbidities such as vasculitis in lupus [2]. How LDNs contribute to the immune response in PwCF is unknown and is the objective of our project. Blood samples were collected from clinically stable adults PwCF with at least one copy of the F508del mutation to isolate NDNs and LDNs by negative selection. We used flow cytometry to determine the proportion, immunophenotype, and antimicrobial functions of NDNs and LDNs and searched for associations with clinical parameters of lung function decline. Sex and age frequency-matched healthy donors (HD) were used as controls. LDNs are composed of mature and immature cells as per the cell-surface expression of CD10 and CD16. The proportion of circulating LDNs in PwCF, specifically the proportion of mature LDNs (n=41; not on modulators) was significantly higher than in HD (n=34). Mature LDNs were associated with more frequent pulmonary exacerbations and with greater lung function decline over time [3]. Circulating mature and immature LDNs remain present in PwCF with highly effective modulator treatment and display distinct antimicrobial functions including a reduced phagocytic capacity, reactive oxygen species production and degranulation in immature LDNs compared to mature LDNs and NDNs. Characterizing neutrophil heterogeneity and contribution to the immune response in PwCF could help to stratify patients into clinically more homogeneous subgroups, improving disease management and understanding of the CF pathogenesis. [1] Rahman S, et al. Ann Rheum Dis 2019, [2] Denny MF, et al. J Immunol 2010, [3] Murru A, et al. J Cyst Fibros 2023.

Lymphocyte, NK cell and mitochondrial gene dysregulation patterns separate patients with neuropsychiatric systemic lupus erythematosus into distinct subgroups with differential anticipated response to targeted therapies

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The aim of this study was to perform an in-depth investigation of the transcriptome of systemic lupus erythematosus (SLE) patients with active central nervous system (CNS) involvement to gain insights into underlying molecular mechanisms and identify new potential drug targets for CNS lupus. We analysed dysregulated gene modules in peripheral blood from patients with active CNS lupus (n=26) and active non-neuropsychiatric (NP) SLE (n=43) versus healthy controls (n=497) from the European PRECISESADS project (NTC02890121). Gene modules were subjected to correlation analyses with serological markers, and regulatory network and druggability analysis. Unsupervised co-expression network analysis revealed 23 dysregulated gene modules. Four showed differential dysregulation between two distinct subgroups of CNS lupus patients. The interferon module was upregulated in both subgroups. In silico prediction algorithms demonstrated a greater anticipated response to anifrolumab and calcineurin inhibitors for the active CNS subgroup with B cell, T cell, cytotoxic/NK cell, and mitochondrial gene downregulation compared with the patient subgroup of mixed dysregulation patterns. In this cohort of SLE patients of European origin, B cell, T cell, cytotoxic/NK cell, and mitochondrial gene dysregulation patterns separated active CNS lupus patients into two distinct subgroups with differential anticipated response to type I interferon and calcineurin inhibition. Our study provides a conceptual framework for precision medicine in CNS lupus.

Maresin Conjugates in Tissue Regeneration (MCTRs) Improve Post-Influenza Pneumococcal Pneumonia by Reprogramming Macrophages and Modulating Inflammation

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Post-influenza (IAV) pneumococcal pneumonia is a major cause of the excess morbidity and mortality seen in IAV epidemics and pandemics. Inflammation triggered by infection is both protective (pathogen clearance) and detrimental (lung injury) during pneumonia. Therefore, the inflammatory response must be finely tuned to ensure a proper response with minimum tissue damage. Specialized proresolving mediators (SPMs), regulate both the innate and adaptive arms of the immune response, and unlike immunosuppressant molecules, SPMs promote host anti-microbial responses. Maresin conjugates in tissue regeneration (MCTRs) are a novel class of SPMs that are produced by and can act on macrophages. The role of MCTRs during respiratory viral infections is not known. Here, we determined that murine IAV infection leads to transient alveolar macrophage depletion and increased susceptibility to infection with *Streptococcus pneumoniae.* Of interest, lung macrophages displayed long lasting phenotypic and transcriptional alterations after macrophage tissue repopulation. After IAV infection, lung macrophages displayed altered expression for pathogen receptors, and pro-inflammatory and host susceptibility genes for pneumococcal infection. Mice exposed to MCTRs (100 ng) after IAV had significantly decreased bronchoalveolar lavage (BAL) leukocytes, especially neutrophils, and reduced BAL fluid total protein levels 48 hours after *S. pneumoniae* infection. Importantly, in addition to decreased lung inflammation, MCTRs significantly decreased lung bacteria counts and substantially decreased bacteremia. Potential mechanisms evident in post-IAV MCTR exposed lung macrophages include increased phagocytosis and expression of CD36, decreased expression of platelet-activating factor receptor and IFN-pro-inflammatory genes. Together, these findings indicate that IAV alters macrophage responses to increase susceptibility for bacterial infection and suggest that macrophage-directed specialized pro-resolving mediators, such as MCTRs, can counter the IAV effects to promote resolution of lung inflammation and enhance host defense.

Metabolic control of virus-induced lung inflammation

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Metabolism and immunity, historically siloed research domains, have recently converged under the concept of immunometabolism.

In the context of influenza virus infection, we have shown that the lung mucosa undergoes metabolic reprogramming, leading to the accumulation of specific metabolites known as metabokines. We revealed the dual functionality of certain metabokines as they regulate host immune cell responses through the modulation of inflammatory signaling and the induction of post-translational protein modifications. Simultaneously, these compounds directly or indirectly disrupt influenza virus replication.

These major findings offer significant potential for innovative treatments targeting inflammation and viruses.

Microbiota is not essential for the development of popliteal lymphatic vessel hyperactivity in the TNF$^{\Delta ARE/+}$ arthritic mouse model

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Alterations in the microbiota can potentially contribute to the onset and severity of rheumatoid arthritis (RA). In this study, we investigated whether the hyperactivity of popliteal lymphatic vessels (pLVs) observed in a specific pathogen-free (SPF) mouse model of TNFα-induced arthritis could be mitigated by germ-free (GF) mice within the same model. pLVs were isolated from male TNF$^{\Delta ARE/+}$ and wild type (WT) SPF and GF 12 week-old mice and mounted on a pressure myograph. Their diameter and contraction frequency (CF) were then measured in response to a stepwise increase in transmural pressure. Effects of the non-selective NOS inhibitor (L-NNA, 100 µmol/l) was evaluated. To confirm inflammation in the TNF$^{\Delta ARE}$ mice, popliteal lymph nodes (pLN) were collected for myeloperoxidase activity (MPO) assessment. RA was evaluated using the Laboras. Results were analyzed by multiple t-test and two-way ANOVA followed by Tukey's test. Pressure myography showed a significant increase in pLV CF (min^{-1}, SPF: 16.2±3.3 vs 8.5±0.5; GF: 11.2±1.2 vs 9.0±0.5, $P<0.05$) and diastolic diameter (µm, SPF 110.3±3.8 vs 97.3±8.1; GF 113.1±4.4 vs 90.7±7.9, $P<0.05$) in TNF$^{\Delta ARE/+}$ mice compared WT. In the presence of L-NNA, the differences in diameters were abolished, while the CF was increased (min^{-1}, 11.0±3.9 vs 6±1.1, $P<0.05$) only in SPF arthritis mice. MPO activity was increased in TNF$^{\Delta ARE/+}$ pLN compared to WT (U/mg, SPF: 0.009±0.001 vs. 0.0005±0.0002; GF: 0.005±0.002 vs. 0.0004±0.00004, $P<0.0001$). Furthermore, Laboras test showed significant decrease in climbing activity (counts, SPF: 9.5±4.5 vs. 279.5±9.9; GF: 33.5±12.2 vs. 298.1 ±55.7, $P<0.05$) in TNF$^{\Delta ARE/+}$ mice compared WT. In summary, our findings confirm that the microbiota does not play a role in the joint-pLV-pLN axis in the development of arthritis but is a factor that worsens the disease. Furthermore, the potential influence of TNF-α on the inflammatory process appears to be associated with changes in the eNOS-NO pathway within the popliteal lymphatic vessel in mice with spontaneous arthritis.

Modulation of Immunometabolism Via NLRX1 Or PLXDC2: Novel Bimodal Mechanisms for the Treatment of Inflammatory Bowel Diseases

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Immunometabolism exerts a bimodal action at the interface of extracellular immune response and intracellular metabolism (Chi. Cell Mol Immunol (19)). Hence, immunometabolic pathways represent an worthy target as a dual checkpoint for the inflammatory cycle. NLRX1 & PLXDC2 have been identified in immunometabolic pathways in multiple cell types in inflammatory bowel disease (IBD, Leber, et al. J Immunol 203(12); Tubau-Juni, et al. J Immunol 206(Supp)). This analysis compares these two key immunometabolic pathways. I*n vitro* murine T cell & macrophage differentiation & *in vivo* mouse DSS colitis models, gene expression, metabolic profiles & cytokine expression were assessed. NX-13, a novel NLRX1 agonist, resulted in regulation of cellular metabolism: activation of mitochondrial genes such as *mt-nd3* & *odgh* and down-regulation of glucose uptake by murine T cells. NLRX1 stabilization by NX-13 increased antioxidant enzyme expression & reduced ROS in T cells. NX-13 specifically reduced effector T cells. These bimodal effects converge to dampened acute DSS colitis (Fig1A). PLXDC2 activation by LABP-69 directly reduced glycolysis, reflected by decreased extracellular acidification in bone marrow-derived macrophages (BMDM) stimulated with lipopolysaccharide as well as reduced superoxide levels in BMDM. PLXDC2 activation downregulated inflammatory cytokines TNFα & IFNγ in T cells. The PLXDC2 agonist PX-04 decreased inflammation in acute DSS colitis in mice (Fig1B). Agents targeting immunometabolism demonstrate innovative potential therapeutic MOAs applicable in IBD. NLRX1 & PLXDC2 represent distinct pathways that modulate the metabolic state simultaneously with inflammation and hence can be targeted to stop chronic inflammation.

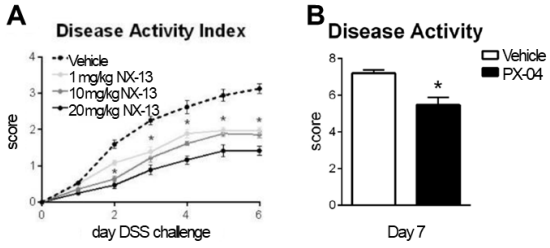

Figure 1: Reduced disease activity in acute DSS colitis caused by the combination of metabolic and immune effects of NLRX1 (NX-13, 0–20 mg/kg) or PLXDC2 (PX-04, 20 mg/kg) activation.

Molecular characterisation based on current geneset databases may not sufficiently define inflammatory processes in conditions with pronounced inflammation: results from the X-HiDE consortium

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The Exploring Inflammation in Health and Disease (X-HiDE) consortium was developed with the aim of unraveling commonalities and differences of inflammatory processes across diseases. Although inflammatory pathways share common attributes across clinical conditions, most conditions are studied separately, resulting in disease-specific drugs and treatment algorithms. However, both broad immunosuppressants and targeted therapies appear effective in multiple conditions. X-HiDE has followed a two-way methodology: (i) characterisation of inflammatory phenotypes determined based on geneset databases (i.e., acute inflammation, low-grade chronic inflammation, immunoparalysis) through curation of publicly available data; and (ii) investigation of inflammatory pathways within and across model diseases. Characterisation of inflammatory phenotypes and identification of model diseases followed a molecular and a clinical approach. Briefly, gene ontology was used as a basis to identify genes of relevance, and each gene and corresponding protein (mediator) were scored as a contributors, inhibitors, or irrelevant for each one of the inflammatory phenotypes. Data curation was independently performed by 6 experts through review of mechanistic studies, with recurrent calibration meetings. Next, relevant diseases were individually scored by 3 clinicians for the presence of components of acute or low-grade chronic inflammation, or resolution. Model diseases were selected, the first ones being sepsis, representative for acute inflammation and immunoparalysis, and systemic lupus erythematosus, representative for acute and low-grade chronic inflammation. Study of the lupus transcriptome pointed to STAT1, PLK1, and B and plasma cell signatures as important for pathogenesis and relevant for drug repurposing, but molecular patterns from cluster analysis were not indicative of the organ manifestations. Another study showed small overlap between geneset enrichment and the molecules identified in each phenotype. These results imply that inflammation is characterised by a multilevel complexity of mechanisms. How inflammatory processes interact with each other remains elusive.

Neutrophil extracellular traps trigger polyclonal B lymphocyte activation independently of antigen specificity toward a pro-inflammatory response

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Activated neutrophils expel neutrophil extracellular traps (NET), DNA/proteins fibers. Described as anti-microbial, NET can become immunogenic. NET formation is increased in rheumatoid arthritis (RA). We have previously shown that NET are pro-inflammatory on macrophages. We suggest that NET act as a DAMP and induce polyclonal B-cell activation, independently of antigen specificity. This mechanism may be amplified in RA patients. Here, we determined whether NET directly activate B lymphocytes, even non-autoimmune and non-memory B cells. **Methods.** Blood neutrophils/PBMC were isolated by density centrifugation from healthy donors (HD)/RA patients. Total or naïve B lymphocytes were purified by magnetic sorting. NET were induced *in vitro* by PMA or ionomycin. B lymphocytes were cultured in the presence/absence of HD/RA NET or LPS/CpG-oligonucleotide. Similar experiments were performed with cells from wild-type and TLR9-deficient mice. Cell purity/phenotype/activation were estimated by flow cytometry. Cytokine/IgG secretion/production were measured by ELISA/flow cytometry. Functional consequences of NET-activated B cells were analyzed on neutrophils/T cells by measuring migration, ROS/cytokine production, proliferation. The pathway triggered by NET was analyzed by RNA-seq. **Results.** NET induced a dose-dependent up-regulation of HLA-DR/co-stimulatory molecules by B lymphocytes both in HD and RA patients and both in total or naïve B lymphocytes. NET-activated B cells proliferate and secreted IL-8, IL-6, TNF, total IgG but not IL-10. The extent of B-cell activation suggests an antigen-independent polyclonal activation. Both HD and RA NET activate B cells, but RA B cells display a stronger response correlated with disease activity. B-cell responses depended on the NET-inducing stimulus. RNA-sequencing revealed NET-mediated up-regulation of RA pro-inflammatory genes and down-regulation of anti-inflammatory genes. Normal and TLR9-deficient B cells respond to NET. Finally, NET-activated B cells trigger neutrophil ROS production/recruitment and T-cell activation. **Conclusions.** NET directly trigger polyclonal B cell activation, even in naïve B lymphocytes, in a TLR9-independent manner, leading to an activated phenotype, a pro-inflammatory cytokine profile and neutrophil/T-cell activation. This mechanism is amplified in RA patients and may be pathogenic.

Neutrophils release glycosaminoglycans to form proteoglycofili and NETs

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Neutrophils play a central role in the host's response to infection, contribute to the inflammatory process and influence cancer development. They communicate with other cells, shaping the immune response through the release of soluble components such as granular proteins, cytokines, and ROS. Additionally, they may release more complex and insoluble components like neutrophil extracellular traps (NETs) or exosomes. Upon deeper investigation, we have identified for the first time the presence of glycosaminoglycans (GAGs) such as chondroitin sulfate (CS) and hyaluronic acid (HA) within NETs. This discovery demonstrates, in an unprecedented manner, that neutrophils are capable of secreting GAGs. Moreover, we have identified a novel DNA-free fibrillar complex, termed proteoglycofili (PGF), which is released by living neutrophils under anoxic conditions, more accurately mimicking the environment of infection and inflammation. We identified that PGF is also composed of granular proteins and both CS and HA. By subjecting PGF and NETs to hyaluronidase treatment, we established that CS and HA are crucial for the maintenance of their filamentous structure and antimicrobial activity. Furthermore, we have demonstrated that the release of these GAGs by neutrophils is dependent on the NADPH oxidase activity, and was shown to be impaired in neutrophils from patients with chronic granulomatous disease (CGD). We have confirmed the secretion of these GAGs by neutrophils, either within PGF or NETs, across various inflammatory disease models, such as mouse colon tumor and mouse prostate adenocarcinoma, and an infectious guinea pig model of shigellosis. Given the broad regulatory role of GAGs in both health and disease, our results strongly indicate that the secretion of GAGs by neutrophils could profoundly influence in the development of many diseases.

New insights into neutrophil-derived myeloperoxidase driven inflammation

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Background: A hallmark of mucosal inflammation is the accumulation of neutrophils (PMN) at sites of injury. Myeloperoxidase (MPO) is the primary antimicrobial enzyme in PMN. MPO generates hypochlorous acid via a reaction between hydrogen peroxide and chloride. Hypochlorous acid diffuses through the tissue and indiscriminately reacts with the phenol moiety in tyrosine to generate chlorinated tyrosine. It is unknown what impact tyrosine chlorination has on cellular function. The current study aims to identify the impact of PMN MPO on the development and resolution of colitis.

Results: Previous studies have shown that therapeutic inhibition of MPO improves acute colitis in a murine model of DSS colitis. We expanded upon these studies and demonstrated that colonic inflammation in MPO KO mice resolved faster than WT mice, as shown by, a decrease in fecal lipocalin and tissue cytokines. Next, we examined the impact of MPO on the development of chronic colitis. MPO KO mice exhibited significantly less inflammation when compared to WT mice. MPO mice had longer colons, lower cytokine levels, and lower histological scores. To better understand the mechanism(s) involved in MPO associated tissue damage and inflammation we extended these studies to include *in vitro* models. Analysis of the extracellular loops of occludin reveals a high number of tyrosine residues, 11 in loop 1 and 7 in loop 2. Given the observation that MPO activation results in the indiscriminate chlorination of tyrosine we examined occludin for 3-chlorotyrosine. Following PMN transepithelial migration and exposure to activated MPO we observed 3-chlorotyrosine within occludin. Further, we then examined the impact of tyrosine chlorination within the binding domain of occludin. It has been reported that a short sequence within the second extracellular loop of occludin is required for tight junction function. Intestinal epithelial cells (IEC) were treated with a short peptide containing this sequence there are significant changes to tight junction length ratio, indicating an impairment of tight junction function. The occludin peptide was neutralized by the chlorination of the tyrosines within the sequence. This suggests that physiologically, the chlorination of tyrosines by PMN MPO disrupts occludin binding. This was further supported by a significant decrease in barrier function in IEC treated with activated MPO.

Conclusions: The results of our study suggests the enzymatic action of MPO promotes chronic inflammation. Activated MPO not only damages tissue and inhibits wound healing, but as shown in our study, also disrupts IEC barrier function. Our work suggests that the barrier dysfunction is a result the chlorination of tyrosines within occludin. Taken together these studies highlight the need to further study the impact of MPO on the inflammatory microenvironment.

PEPITEM switches off the production of pro-inflammatory mediators to limit leukocyte trafficking into the inflamed joint.

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Purpose: The adiponectin-PEPITEM pathway negatively regulates leukocyte infiltration into inflamed tissues, but this immunomodulation is lost in patients with established treated RA. Here we investigate the status of the adiponectin-PEPITEM pathway in early treatment naïve arthritis patients and whether replacing it may have clinical benefit.

Methods: Adiponectin receptors were measured by qPCR or flow cytometry on peripheral blood lymphocytes (PBLs) from healthy donors or patients presenting to early arthritis clinics with undifferentiated arthritis or treatment naïve RA. Mice with collagen-induced (CIA), antigen-induced (AIA) or gouty arthritis where treated daily with PEPITEM or vehicle control. Disease severity and joint swelling were assessed daily. Joints were digested and assessed by flow cytometry to determine leukocyte infiltration or by scRNAseq of sorted $CD45^+$ cells, ELISA or western blot analysis for gene or protein expression.

Results: Adiponectin receptor gene and protein is significantly reduced in leukocytes from patients with untreated RA compared to patients with resolving arthritis or healthy controls.

In vivo, PEPITEM significantly reduced disease severity, joint swelling and leukocyte infiltration (including T-cells and monocyte/macrophages) in CIA, AIA and gouty arthritis models, when compared to vehicle treated mice. Using the inflamed AIA joints, scRNAseq analysis revealed notable decrease in genes linked with chemokine activity and cell chemotaxis in M1-like macrophages in PEPITEM treated animals compared to vehicle controls, whilst the converse was true for the M2-like macrophage cells. These data were confirmed at protein level, where significant reductions in several pro-inflammatory cyto/chemokines (e.g., JE (CCL2), KC (IL-8), TNFα, IL-6) were seen in the joints of PEPITEM treated mice compared to controls. Moreover, significant modulation of COX2 and NF- κB signalling pathways were also detected in animals treated with PEPITEM. *In vitro*, PEPITEM significantly reduced TNFα and IL-6 production by macrophages.

Conclusions: Patients with RA with defects in the adiponectin-PEPITEM pathway would potentially benefit from PEPITEM replacement therapy. Preclinical murine studies demonstrate PEPITEM modulates leukocyte trafficking within inflamed joints by switching the synovium microenvironment from a pro-inflammatory to a more pro-resolution state. Thus, PEPITEM may offer an alternative therapy alone or in combination with other biologics for early RA patients to reset the synovial microenvironment.

Pharmacological Evidences That the Inhibitory Effects of Prostaglandin E_2 Are Mediated by the EP_2 and EP_4 Receptors in Human Neutrophils

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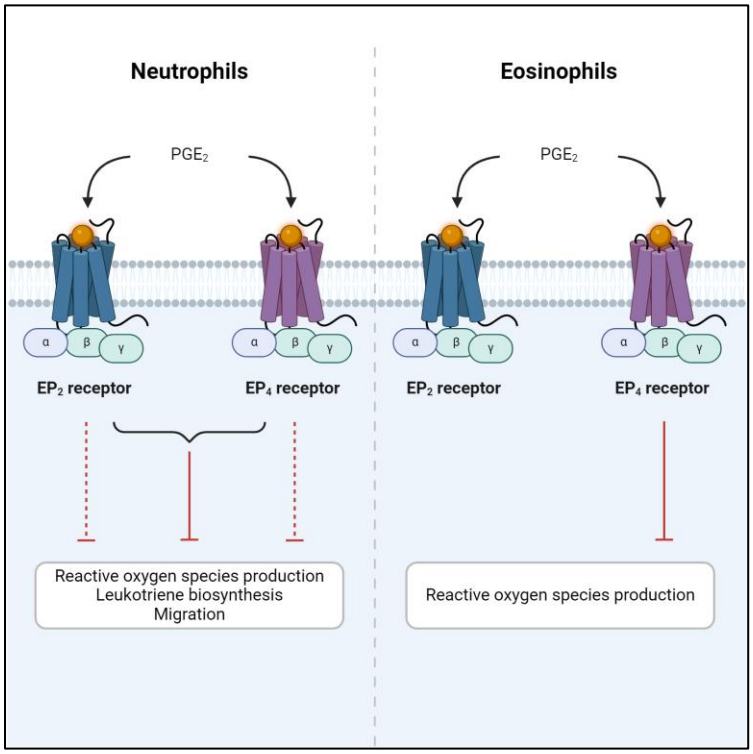
Prostaglandin (PG) E_2 is a recognized inhibitor of granulocyte functions. However, most of the data supporting this was obtained when available pharmacological tools mainly targeted the EP_2 receptor. Herein, we revisited the inhibitory effect of PGE_2 on reactive oxygen species production, leukotriene biosynthesis and migration in human neutrophils. Our data confirm the inhibitory effect of PGE_2 on these functions and unravel that the effect of PGE_2 on human neutrophils is obtained by the combined action of EP_2 and EP_4 agonism. Accordingly, we also demonstrate that the inhibitory effect of PGE_2 is fully prevented only by the combination of EP_2 and EP_4 receptor antagonists, underscoring the importance of targeting both receptors in the effect of PGE_2. Conversely, we also show that the inhibition of reactive oxygen species production by human eosinophils only involves the EP_4 receptor, despite the fact that they also express the EP_2 receptor.



Potential of Protectin DX analogues as novel antidiabetic therapeutics

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Obesity is characterized by chronic low-grade inflammation which promotes numerous complications such as type 2 diabetes (T2D) and metabolic dysfunction associated fatty liver disease (MAFLD). A class of lipid mediators known as Specialized Pro-resolving Mediators (SPMs) has garnered interest in this field due to their capacity to promote inflammation resolution pathways. Some SPMs also appear to act directly on inflammation disrupted biological functions to aid in the return to homeostasis. One such SPM is Protectin DX (PDX), the stereoisomer of Protectin D1 (PD1). Our group previously demonstrated that PDX ameliorates metabolic inflammation and glycemic control and attenuates end-stage renal failure in two different T2D murine models. Our group recently developed cost-efficient synthetic routes for PDX and structural analogues to accelerate research on PDX functions and to scale-up production of these molecules for preclinical therapeutic studies. Thus, over 30 PDX analogues were synthesized and screened to evaluate their bioactivity on relevant cellular models. Fifteen of these small PDX structural mimics reduced inducible nitric oxide synthase (iNOS) activity in LPS treated J774 macrophages. Six analogues, but not PD1, increased glucose uptake in L6 and C2C12 myocytes. None of them, however, replicated PDX and PD1 capacity to activate PPARγ transcriptional activity in a cell-based luciferase reporter assay. A few bioactive analogues were next tested *in vivo* for their ability to suppress LPS-induced inflammation in golden Syrian hamsters. One analogue, RM-598-48 was found to significantly decrease plasma TNF-α, specifically in female hamsters. Lastly, a low dose of RM-598-48 (50 ng/g) orally administered daily was found to reduce hepatic steatosis and to prevent fasting hyperinsulinemia in hamsters fed an obesogenic high fat high fructose mixed protein diet. These results suggest that cost-efficient analogues of PDX display a high potential as new therapeutic drugs against obesity-linked inflammation, T2D, and MAFLD.

Pro-resolving lipid mediators prevent cancer cachexia

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Most patients with advanced cancer suffer from cachexia, which results in the death of 20% of patients. There are no approved therapies for cancer cachexia as the underlying mechanisms remain poorly characterized. The hallmark of cachexia is unresolved hyperinflammation resulting in a devastating muscle wasting syndrome. Cachexia-induced apoptotic cell death occurs in many tissues by pro-inflammatory cytokines. A paradigm shift is emerging in understanding the resolution of inflammation as an active biochemical process with the discovery of novel specialized pro-resolving mediators (SPMs). SPMs stimulate clearance of debris, promote muscle regeneration, and counter-regulate cytokines. We hypothesized that cachexia results from disrupted resolution of inflammation. We profiled lipid mediators in cachexia models via state-of-the-art targeted metabololipidomics. Here, we've identified dysregulated SPMs in six cancer cachexia models. SPMs (e.g., RvD2 and MaR1) were markedly reduced in colon cancer (CT26)-induced cachectic mice on day 35 post-tumor cell injection vs. non-tumor bearing mice (NTB). SPMs (RvD1, RvD2, LXA_4, and MaR1) were also dramatically dysregulated in Lewis lung carcinoma (LLC)-induced cachectic mice, including the gastrocnemius and tibialis anterior muscles, heart, liver, and spleen, on day 20 post-tumor cell injection. Cachexia induced a pro-inflammatory eicosanoid storm in plasma from CT26-induced cachectic mice. Chemotherapy also induced cachexia *via* loss of SPMs in a lymphoma (EL4) and ovarian cancer (ID8) mouse model. At 10 days post-LLC tumor resection, the RvD1 receptor (ALX/FPR2) KO and RvE1 receptor (ChemR23/ERV) KO mice exhibited 20-23% loss in body weight compared to WT mice. Thus, cancer cachexia is resolvin receptor-dependent. RvD2 and PCTR2 prevented LLC- and B16F10 melanoma-induced cachexia at 15 nanograms/day compared to control without immunosuppression. RvD2 prevented pancreatic cancer (KPC)-induced loss of grip strength and prolonged survival compared to control. In contrast to celecoxib, SPMs prevented the cachexia-induced cytokine storm, including inhibition of TNF-α, CCL2, CCL3, CCL4, CXCL2, G-CSF, and PAI-1. SPMs were sharply reduced by up to 85% in the plasma of pancreatitis patients at risk for cachexia compared to healthy individuals. Thus, our studies shall provide the basis for the clinical translation of SPM-directed treatments in humans as a new direction to potentially prevent and/or reverse cancer cachexia.

Product impurity takes its toll: The actual ability of Toll-like receptors to generate neutrophil extracellular traps (NETs)

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Neutrophils are usually the first cells recruited to sites of injury or infection, where they exert several effector functions to protect the host, including the release of NETs. These extracellular webs of decondensed chromatin adorned with antimicrobial molecules can trap and inactivate pathogens. The presence of injury or infection can be detected via Toll-like receptors (TLRs). Neutrophils express TLR1, TLR2, TLR4, TLR5, and TLR6 on their surface, as well as TLR7, TLR8, and TLR9 on their endosomes. Here we dissect which TLRs can promote the formation of NETs using ultrapure ligands. We found that in humans NETs are only induced following TLR2 ligation, whereas TLR4, TLR5, TLR7, TLR8, and TLR9 engagement fail to do so (but do stimulate other cellular responses as expected). A widely used (but only partially purified) lipopolysaccharide preparation induced NET formation by binding TLR2 (and possibly other surface receptors), confirming data from several other groups who used the same LPS preparation and concluded that TLR4 ligation promotes NET generation by human neutrophils. By contrast, murine NETs are formed upon both TLR2 and TLR4 engagement using ultrapure ligands. We next investigated the molecules downstream of TLR2 involved in NET production in humans. Similar to other classes of physiological NET inducers, TLR2 ligand-triggered NET formation is controlled by signaling kinases (TAK1, MEK, p38 MAPK) mobilized early during this response, while Syk, PI3K, and PLCγ2 acted belatedly (+2h after stimulation). Likewise, endogenous factors are released during TLR2-induced NET formation and feed back to bind the RAGE receptor, thereby sustaining the NET response. Finally, PAD4 proves to be essential to NET production following TLR2 ligation, while elastase appears to be dispensable. Unlike other physiological NET inducers, TLR2-induced NET formation partially relies on reactive oxygen species production. Our study shows the surprisingly restricted repertoire of TLRs that can elicit NET formation in humans and illustrates how this emblematic neutrophil response differs between humans and rodents.

Proteases-Activated Receptors play a key role in postoperative ileus

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Postoperative Ileus (POI) is an iatrogenic complication characterized by a transient paralysis of gastrointestinal motility. This transient paralysis leads to food intolerance, nausea, vomiting in patients, and thus prolongs hospitalization time. The underlying mechanisms are described in two phases: a neurogenic phase followed by an inflammatory phase. It has been shown that immune cells such as macrophages and mastocytes are able to produce numerous proteases and releasing them into their environment upon activation. Proteases, in turn, could activate Protease-Activated Receptors (PARs) involved in numerous inflammatory pathways. Therefore, we developed a mouse surgical model of Postoperative Ileus to explore the relationship between this iatrogenic complication and proteolytic activation of PARs.

To address this question, *PAR-1*, *PAR-2*, or *PAR-3* knockout mice were subjected to POI protocol. Additionally, a pre-treatment with PAR antagonists was performed two hours before surgery on wild type (WT) mice. Gastric emptying and small intestine motility were assessed using a charcoal solution. Pro-inflammatory cytokines levels were evaluated by qPCR and ELISA. Immune cells recruitment was measured by flow cytometry.

Mice with POI showed no gastric empty and slower gastrointestinal transit compared to mice subjected to laparotomy (sham condition). POI mice exhibited a significant increase mRNA levels of pro-inflammatory markers (*CCL2, IL6, TNFa, ICAM-1*) in the ileum muscularis externa 2.5 hours after intestinal manipulation compared to sham mice. Additionally, 24 hours post-surgery, mice showed an increase in the chemokine CCL2 in the ileum muscularis externa, as well a recruitment of immune cells including neutrophils, monocytes, and monocyte-derived macrophages. Based on this model of POI, PAR-1 and PAR-3 receptor-deficient mice were not protected from Postoperative Ileus. In contrast, PAR-2 receptor-deficient mice subjected to POI model exhibited a gastric emptying and a normal GI transit. Furthermore, the pre-treatment with PAR-2 antagonist (GB88 at 10uM) protected WT mice from POI.

These results highlight a new player in development of Postoperative Ileus: PAR 2, which could be considered as a new pharmacological target.

https://www.conferium.com/convPages/conv_240.lasso?papermanagement=true&refNo=240-ZSKv-162&pass=Uf86i&lang=en

Proteolytic Activity Correlates with Tissue Permeability & Symptom Status in Crohn's Disease

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Objective: Dysregulated fecal and epithelial proteolytic activity (PA) has been detected in patients with inflammatory bowel disease (IBD) versus healthy controls. Recently, increased fecal PA that preceded diagnosis of UC was found to have a microbial component that caused inflammation in mice, suggesting it could be used as a luminal diagnostic biomarker in IBD. The aim of this study was to investigate whether PA in IBD patients correlates with disease activity. **Methods**: 23 adult CD patients were recruited at McMaster Children's Hospital and biopsies were collected from non-inflamed and proximal (within 5 cm) inflamed sites from the same patients. Luminal to serosal flux rate and tissue conductance were measured in biopsies with Ussing chambers. Total proteolytic (trypsin), elastase-like (FITC-elastin and N-Suc-Ala3-pNA), and mucolytic (bioassay) activities were also measured at both sites. In a separate cohort of 16 CD and 19 UC patients recruited by the IMAGINE study (imaginespor.com), overall, elastase-like, and mucolytic fecal PA were measured. IMAGINE patients completed the Symptom IBD Short Index (SIBDSI) survey to classify patients as asymptomatic (UC <13, CD <14) or symptomatic (UC >13, CD >14) at the time of fecal donation. Fecal calprotectin (fCal) levels were measured to determine remission (fCal<50μg/g) or flare (fCal>100μg/g) status of patients. **Results**: There was higher paracellular permeability and tissue conductance in biopsies from inflamed areas compared with non-inflamed areas from the same patient. Elastase-like and mucolytic activities were higher in inflamed tissue compared with matched non-inflamed ($p<0.05$ and $p<0.04$, respectively). In IMAGINE patients, total fecal PA was higher in symptomatic compared with asymptomatic CD patients ($p=0.04$). There was a trend for higher overall proteolytic activity in IBD patients categorized in flare, or fCal>100μg/g, compared with patients in remission, or fCal<50μg/g. **Conclusions**: There is growing evidence for the role of host and microbial PA in the progression and exacerbation of IBD. Tissue permeability and elastase-like activity correlate with inflammation and may be causally linked. The results raise the hypothesis that microbial elastase-like activity may cause mucosal inflammation through barrier disruption and constitute a novel non-invasive biomarker for monitoring CD activity.

Regulation of Macrophage Activation and Disease Pathogenesis following Ozone Exposure by Extracellular Vesicles and miRNA Cargo

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Exposure to air pollutants like ozone contributes to the pathogenesis and severity of inflammatory diseases including asthma, emphysema, and ARDS. We previously demonstrated that lung injury following of ozone is due, not only to its direct effects on the lung, but indirectly to the actions of inflammatory mediators released by activated macrophages; moreover, macrophage activation is controlled, at least in part, by epigenetic regulators particularly, noncoding microRNAs (miRNA)s. Extracellular vesicles (EVs) are cell-derived particles that facilitate cell-cell communication by delivering cargo, including miRNAs, from donor to recipient cells. We found that miRNA cargo released from lung cell EVs after acute ozone exposure (0.8 ppm, 3 h) regulates macrophage-mediated inflammatory responses. Herein, we analyzed the EV-cargo miRNA profiles isolated from bronchoalveolar lavage fluid (BAL) after chronic ozone exposure. Mice (C57Bl6/J, 11-12 wk) were exposed to air or ozone (1.5 ppm, 2 h), 2x/wk for 6 wk. BAL was collected 24 h after the last exposure, EVs isolated and analyzed by flow cytometry to assess their origin. Ozone exposure resulted in histo-pathologic changes in the lung consistent with inflammation and chronic disease. In both control and ozone treated mice, BAL EVs originated mainly from $CD45^+$ macrophages, with a smaller % from $CD326^+$ epithelial cells and $CD31^+$ endothelial cells. Ozone had no effect on the origin of the EVs, or absolute numbers of EVs that originated from these cells. Next, we assessed the effects of ozone on EV-cargo miRNA and their pre-miRNAs precursors by RNA sequencing. Among the 1,935 miRNAs detected in BAL EVs after ozone exposure, 8 miRNAs were found to be significantly upregulated (more than 2.85-fold change) relative to EVs from mice exposed to air control. We also identified 3 pre-miRNAs which were significantly altered (1.5 - 2 fold) after ozone exposure. All of these miRNAs and pre-miRNAs are newly identified, and their function and regulatory activity are unknown. These findings are important as they suggest novel pathways mediating macrophage activation, which can be targeted to reduce lung injury and chronic disease.

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Relationship of serum levels of inflammatory marker endocan with apoptosis and severity of atherosclerotic coronary artery lesions

A.V. Kubyshkin, E.A. Zakharyan

Endocan was found more than 20 years ago, and this biomarker for inflammation and endothelial dysfunction is still being actively researched. Sometimes referred to as endothelial cell-specific molecule-1 (ESM-1), endocan is a soluble dermatan sulfate proteoglycan produced by endothelial cells. It is expressed in actively proliferating tissues and detected in cultured endothelial cells of the skin, fatty tissue, hepatocytes, pulmonary and coronary arteries, etc. The role of endocan was studied in many diseases associated with inflammation and endothelial dysfunction, such as type 2 diabetes mellitus, arterial hypertension, atherosclerotic cardiovascular diseases, kidney diseases, obesity, polycystic ovary syndrome, metabolic syndrome, non-alcoholic fatty liver disease, sleep apnea syndrome. Given the known association of inflammatory and apoptotic processes of atherogenesis, it would appear relevant to study the relationship between endocan concentration and apoptosis marker levels in patients with coronary artery disease (CAD) within clinical examination and laboratory test context.

Objective. Explore the relationship of serum concentration of endocan as a marker of inflammation with the indicators of apoptosis and clinical and laboratory characteristics of patients with CAD.

Material and Methods. The study included 264 people (161 males and 103 females), of whom 220 patients had documented CAD. Anthropometric measurements, coronary angiography, echocardiography, duplex ultrasound scanning of extracranial parts of the brachiocephalic arteries were performed for all patients. Concentrations of endocan (ng/ml) and apoptotic markers Bcl-2 (ng/ml), Bax (ng/ml), Bcl-2/Bax, TRAIL (pg/ml) p53 (ng/ml) were measured in blood serum. Patients were divided into groups based on their SYNTAX scores: group 1 with moderate atherosclerotic lesions of the coronary arteries (CA) (score < 22, 124 patients); group 2 with severe CA atherosclerosis (score 23-32, 53 patients); and group 3 with extremely severe CA lesions (score >33, 43 patients). The control group consisted of healthy volunteers (44 subjects). All groups were age- and sex-matched. Differences were considered statistically significant at $p<0.05$.

Results. A correlation was found between endocan concentration and CAD severity ($r=0.32$, $p<0.001$). In group 1, the median endocan concentration was 14.40 ng/ml [10.19; 19.91], in group 2, 20.31 ng/ml [12.75; 24.12], in group 3, 32.10 ng/ml [22.12; 38.21] and in the control group, 5.97 ng/ml [4.38; 8.25] ($p<0.0001$). Correlations of varying strength and significance were observed between the endocan concentration and a number of clinical and instrumental characteristics. Endocan concentrations significantly differed in groups of patients with multivessel disease ($p<0.01$), angina pectoris ($p<0.0001$), a history of myocardial infarction ($p<0.001$), and obesity ($p<0.05$) from patients without these signs. Also, a correlation was found between serum endocan concentration and apoptotic markers: TRAIL ($r= -0.524$, $p<0.0001$); BCL-2 ($r= -0.558$, $p<0.0001$), Bax ($r= 0.571$, $p<0.0001$), Bcl-2/Bax ($r= -0.599$, $p<0.0001$) and p53 ($r= 0.560$, $p <0.0001$).

Conclusion. The international scientific community has been actively searching for novel laboratory markers of the progression of atherosclerosis processes in recent decades. Attention is paid to the study of the relationship between inflammatory and apoptotic mechanisms of atherosclerotic plaque formation and evolution, including the presence of delay, defective phagocytosis, and active release of pro-inflammatory mediators and signaling molecules in hyperlipidemia. We found that there is a statistically significant increase in serum concentrations of the inflammatory marker endocan in patients with CAD as coronary atherosclerosis becomes more severe. We also revealed correlations of varying strengths between the levels of endocan and some clinical examination and laboratory indicators. The data obtained suggest that endocan can be used as a diagnostic marker of the severity of atherosclerotic processes in patients with CAD.

Resolvins enhance immunotherapy to induce Fanconi anemia tumor regression via inflammation resolution

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Fanconi anemia (FA) is a condition distinguished by early-onset solid tumors, showing resistance to conventional cancer treatments such as chemotherapy and radiation. A major feature of FA is chronic inflammation, though the mechanisms governing the emergence and resolution of inflammation in FA remain poorly understood. We hypothesized that dysregulated resolution metabolomes and a systemic pro-inflammatory eicosanoid storm of inflammation-initiating mediators control FA tumor progression. To investigate we utilized a new murine model of transplantable FA tumors, focusing on head and neck squamous cell carcinoma (HNSCC) lacking the Fancc gene. We found that the advancement of Fancc-deficient HNSCC disrupts the resolution of inflammation by unbalancing specialized pro-resolving lipid mediators (SPMs) and eicosanoids. Tumor-bearing mice lacking Fancc demonstrated an increase in eicosanoids, notably leukotriene B 4 (LTB 4) and thromboxane B 2 (TXB 2) in the spleen alongside a 113% prostaglandin E metabolite (PGEM), a marker of PGE 2 production, in plasma compared to non-tumor-bearing (NT) mice. The non-tumor-bearing mice exhibit a decline in SPM/LTB 4 ratio, including RvD1/LTB 4, RvD2/LTB 4 , and MaR1/LTB 4 compared to NT mice. The activity of the immune checkpoint blockade in tumors deficient in mismatch repair, including head and neck cancers, indicates a relevance for immunotherapy in FA-induced cancers. Notably, individuals with FA maintain preserved levels of absolute T cells and CD4+T cell function. In a novel murine FA tumor model, we combined stimulation of the resolution of inflammation (i.e., treatment with RvDs) with immunotherapy. While treatment with RvD4, RvD5, or anti-PD1 immunotherapy alone delayed tumor growth compared to control at treatment day 20, monotherapy failed to prevent tumor escape by treatment day 30. Remarkably, combining RvD4 or RvD5 with anti-PD1 immunotherapy resulted in sustained regression of Fancc-/-tumors. Furthermore, RvD4 and/or anti-PD1 immunotherapy inhibit the growth of orthotopic Fancc-/- tumors compared to control. The coaction of resolvins and immune checkpoint blockade resulted in Fancc-/- tumor regression through mechanisms such as macrophage phagocytosis of apoptotic debris, counter-regulation of pro-angiogenic cytokines, and the inhibition of angiogenesis. By antagonizing Triggering Receptor Expressed on Myeloid cells-2 (TREM2) in conjunction with resolvins and immunotherapy, we restored SPM/eicosanoid ratios in Fancc-/- tumor-bearing mice to levels observed pre-cancer. SPMs and eicosanoids, therefore, present as potential early biomarkers and biological targets in FA-induced cancer progression. The stimulation of inflammation resolution via pro-resolution lipid mediators to bolster immunotherapy represents an innovative host-centric therapeutic approach, aimed at preventing FA-induced cancer progression through debris clearance and cytokine suppression. These results underscore the significance of targeting

inflammation resolution via resolvins to enhance immunotherapy in the prevention and reversal of FA-induced cancer progression

Role of IL-17RD in Antiviral Innate Immunity

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Cytokines, such as IL-17A and IL-17F, are pivotal in immune responses, with Th17 cells and innate immune cells producing these proteins. Their involvement extends beyond anti-infectious and anti-fungal responses and is linked to inflammatory conditions like psoriasis. The IL-17 receptors family is composed of IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE, They trigger a cascade of events leading to the activation of immune cells. Regulatory mechanisms exist to prevent excessive immune responses. Notably, IL17RD displays anti-inflammatory activities in part believed to be mediated through its negative impact on signaling cascades including the mitogen-activated protein kinases (MAPKs) and toll-like receptor-dependent pathways. Recognizing IL-17RD as a potential antiviral repressor, our study aims to elucidate its function. Specifically, we hypothesize that IL-17RD is a repressor of antiviral innate immune responses following RNA virus infection, influencing the type I/III interferon (IFN) responses. Using IL17RD-deficient A549 pneumocytes as a cellular model, we demonstrate that the melanoma differentiation-associated protein 5 (MDA5) signaling pathway is hyperactivated, resulting in an upregulation of key inflammatory genes, including *IFNβ*, *IFNL3*, *CCl5*, and *IL6* as observed through qPCR. Additionally, western blot analysis revealed a more robust activation of the MDA5 pathway, indicated by increased in the activation of interferon-regulatory factor 3 (IRF3), in the absence of IL-17RD. Conversely, overexpression of IL17RD led to decreased IRF3 activity in response to MDA5 engagement. Moreover, silencing IL17RD in the SARS-Cov2 cellular model, A549-ACE2 resulted in the exacerbation of numerous inflammatory genes. IL-17RD expression profiles will be next examined in severe COVID-19 patients, offering insights into its relevance in real-world viral infections. Understanding IL-17RD's role as an antiviral repressor holds significant promise. It could pave the way for targeted treatments, potentially reducing its expression in immunocompromised patients and strengthening their immune defenses against viral infections. This research advances our understanding of antiviral with potential implications for mitigating the severity of viral infections across diverse populations, including addressing challenges posed by viruses such as COVID-19.

Role of IRG1 in Regulating Inflammatory Signaling

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Sepsis is a potentially fatal condition which arises when the body's response to an infection is overly excessive, keeping it in an unbalanced state. Sepsis is often associated with the patients experiencing organ dysfunction resulting in death. Similarly, a majority of those who survive from sepsis suffer long-term sequelae. Identification and understanding of the genes involved in inflammation will help tackle the problem of patient care, sepsis prevention and help decrease the mortality rates. During inflammation certain genes in the body are activated trying to clear out the infection and bring the immune state back into balance. Regulation of specific genes aid the production of signaling proteins which help control the ongoing inflammation. Signaling proteins can be either proinflammatory or anti-inflammatory. Host response to infectious agents trigger immune responses to aid the elimination of pathogens.

Immunometabolism encompasses multiple regulators including transcription factors and signaling proteins. This project focuses on a specific gene (IRG1) that is upregulated during inflammation. The inflammatory state is induced using stimulants such as lipopolysaccharide (LPS) to emulate sepsis-like condition. The project utilized *in vivo* and *in vitro* methods to investigate expression levels in innate immune system cells. The results showed increased expression of IRG1 gene upon LPS stimulation. The increase in the expression levels was more apparent over time. Under the same conditions cytokine analysis detected increased levels of proinflammatory cytokines in the cells with overexpressed gene compared to the cells with the IRG1 knockout gene. Survival studies in mice were conducted in order to investigate the importance of the gene in animal models. The results showed increased survival rate in mice where the gene was deleted compared to the wild type mice. This study established IRG1 as a potential therapeutic target for bacterial infections.

Role of neutrophil proteinases in the local and systemic inflammation development

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Neutrophils take important part in nonspecific defense of organism by mechanism of phagocytosis. But also, very important role neutrophils play in processes of exocytosis and formation of "extracellular traps". In experimental and clinical studies, the role of nonspecific neutrophil proteinases (elastase-like & trypsin-like activity) and their endogenous inhibitors (antitrypsin activity & acid-stable inhibitors) in the development of local inflammation in the lungs, endometrial hyperplasia (EH) and systemic inflammation in critical conditions was studied.

The results showed that the development of local inflammation leads for nonspecific reaction of proteinases and their inhibitors in the blood serum with increase of proteinases and their inhibitors levels. Investigation of the bronchoalveolar lavage fluid showed more specific changes, which are characterized by a phase reactions: in the acute period the changes can be characterized as compensatory, in chronic – the level of proteinases increases with a decrease activity of local inhibitors. In the patients with EH we detected growth in 3-5 times of elastase- and trypsin-like activity in the uterine lavage fluid from simple form to atypical EH with decrease level of inhibitors.

In critical conditions, both at the local and systemic levels, an imbalance develops in the proteinase-inhibitor system, which is characterized by a decrease activity of the antiproteinase potential and increase activity of proteinases in period within 24-48 hours of decompensated stage of shock.

These studies made it possible to characterize the types of reactions of nonspecific proteinases and their inhibitors at the systemic and local levels. At the blood level, compensated and decompensated types of reactions were described. At the local level, 4 types of changes are described, which can be characterized as potentiated, compensated, destructive and decompensated. Depending on the types of reactions, it is possible to evaluate compensatory potential and decide on the use of proteinase inhibitors for therapeutic purposes. In addition, in critical conditions, the assessment of the proteinase-inhibitory balance made it possible to propose an original classification of shock: with primary and secondary formation of the systemic inflammatory response syndrome (SIRS).

Sex-specific impact of early life stress on lung inflammatory response in adults.

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Introduction: Stress influences the immune system according to the nature, intensity, and time during which stress is experienced. It is well established that the consequences of stress on developing brains and cortisol production are sex-specific. However, there is limited knowledge on the long-term impact of early life stress on immune responses. Given that immune programming occurs early in life and that immunity and stress both involve sexual dimorphisms, our hypothesis is that early life stress induces sex-specific immune alterations.

Objective: Our objective is to evaluate the sex-specific impact of early life stress in adults on lung immune response.

Methods: We used a well-established rat model of early life stress: neonatal maternal separation (NMS). Lung antimicrobial inflammatory responses to either Poly I:C or LPS were assessed in adult female and male control (CTRL) and NMS. Innate and adaptative immune cells from broncho-alveolar lavage (BAL) of adult rats were identified using flow cytometry.

Results: We observed that activation of adaptative immune responses (including B and T cells) is stronger in female NMS compared to controls. Interestingly, NMS increased BAL cell recruitment after LPS exposure, especially in male NMS, whereas after Poly I:C exposure, we observed lower recruitment of cells in BAL, markedly in female NMS. Moreover, the proportion of alveolar macrophages was higher in female NMS compared to male NMS after LPS exposure only. On the other hand, we demonstrated that following LPS exposure, male NMS had an increased neutrophilic inflammation whereas following Poly I:C exposure, neutrophilic inflammation appears in female NMS.

Conclusion: These results suggest that anti-viral and anti-bacterial responses are sex-specifically altered by NMS, even though corticosterone dysregulation after NMS is only observed in males. The impact of NMS on long-term immune response could originate from sex-specific alteration of bone-marrow immune progenitors.

Skin innate immune response to Usutu virus infection

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Usutu virus (USUV) is an emerging arbovirus belonging to the *Flavivirus* genus transmitted to the host during a mosquito's blood meal. The infection can be asymptomatic or lead to meningoencephalitis in humans. Following the bite of an infected mosquito, the skin represents the initial site of inoculation of this virus and provides the first line of host defense. Currently, few data are available on the interactions between skin cells and USUV as well as on the inflammatory and antiviral responses induced. In order to study the pathophysiology of USUV skin infection, the permissivity of primary resident skin cells to USUV infection and the pathways involved in viral recognition and innate immune response activation were characterized. Our results showed an early viral replication during the first 24 hours in human epidermal keratinocytes, dermal fibroblast and skin explants leading to the induction of antiviral and pro-inflammatory targets. *In vivo*, following cutaneous inoculation, we demonstrated that USUV can rapidly spread, replicate and persist in all distal cutaneous tissues in mice, a phenomenon associated with a generalized skin inflammatory response. Moreover, a main role of the RIG-I receptor in USUV sensing, induction of the inflammatory and antiviral response and restriction of the infectious viral particle production was shown. Finally, type I interferon signaling has been shown to be essential to prevent USUV dissemination to the peripheral organs. Together, these data provide a better understanding of the pathophysiology of the early stages of USUV infection and highlight the key amplifying and immunological role of the skin during USUV infection.

Stereocontrolled Total Syntheses of Resolvin-Epoxide Intermediates and their Transformation to Potent Pro-resolving Mediators by Human Leukocytes

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A growing body of evidence indicates that specialized pro-resolving mediators (SPMs) play a pivotal role in the resolution of inflammation (*Nature*, **2014**, 510:92-101). Recently, we achieved the first total synthesis of 4S,5S-epoxy-17S-hydroxy-6E,8E,10Z,13Z,15E,19Z-docosahexaenoic acid (*RSC Adv*., **2022**, 12:11613-18), the biosynthetic precursor to potent resolvin D3, resolvin D4 and 4S,5R-RCTR1. We also accomplished the total synthesis of resolvin 7,8(S,S)-epoxytetraene and are currently investigating its critical role in natural formation of pro-resolving mediators. Stereospecific and chiral pool-based synthetic approaches were employed, with key synthetic features including Sonogashira coupling, Sharpless asymmetric epoxidation, and a late-stage Wittig olefination to forge the core carbon skeleton. Next, we demonstrated experimental evidence supporting the role of this highly labile epoxide ($T_{1/2} \approx 5$ sec; aq. solutions) in the biosynthesis of resolvin D3 (4S,11R,17S-trihydroxy-5Z,7E,9E,13Z,15E,19Z-docosahexaenoic acid) and resolvin D4 (4S,5R,17S-trihydroxy-6E,8E,10Z,13Z,15E,19Z-docosahexaenoic acid) by human neutrophils and M2 macrophages (*PNAS*, **2021**, e2116559118). Of interest, this transient epoxide was also converted to a novel cysteinyl-resolvin, 4S,5R-RCTR1 (5R-glutathionyl-4S,17S-dihydroxy-6E,8E,10Z,13Z,15E,19Z-docosahexaenoic acid), by M2 macrophages. Both resolvins D3 and D4 are potent signaling molecules with protective actions, *i.e*., limit neutrophilic transmigration and enhance macrophage phagocytosis and efferocytosis, as well as modulate the severity of deep vein thrombosis (*Nat Rev Cardiol*., **2024**, PMID: 38216693). Total synthesis of the newly uncovered cys-resolvin enabled its structural elucidation and biological activity studies including clearance of live bacteria and erythrophagocytosis of senescent human red cells (*Am. J. Hematol*., **2023**, 98:1000-16). Together, these results confirm the role of 4S,5S-epoxy-resolvin intermediate in the biosynthesis of bioactive resolvins D3, D4 and 4S,5R-RCTR1 in a cell-type specific manner. Currently, biosynthetic studies involving these allylic epoxide intermediates are underway and results will be presented. The authors gratefully acknowledge support by US NIH grants P01GM095467 and R35GM139430.

Suppression of Adipocyte ABHD6 in Mice Promotes Healthy Expansion of Adipose Tissue in Obesity

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Excessive accumulation of visceral fat in obesity often leads to insulin resistance, inflammation, type 2 diabetes and cardiovascular diseases. However, some obese individuals with obesity show healthy expansion of white adipose tissue (WAT), without cardiometabolic complications, and such obese condition is named metabolically healthy obesity (MHO). Global deletion of the monoacylglycerol (MAG) hydrolase, α/β-hydrolase domain-containing 6 (ABHD6) has revealed the therapeutic potential of ABHD6 inhibitors against obesity, yet the immunometabolic role of adipocyte ABHD6 in WAT expansion and energy balance in obesity is not known. We now show that while inducible adipocyte-specific ABHD6-KO (AA-KO) mice become obese upon high-fat diet (HFD) feeding, they do not display insulin resistance, fatty liver, and inflammation. AA-KO HFD-fed mice, show healthy expansion of visceral fat with smaller adipocytes, curtailed inflammation, and elevated lipolysis and fatty acid oxidation. AA-KO adipocytes-conditioned medium but not that from control mice promotes preadipocyte differentiation and anti-inflammatory polarization of WAT macrophages. We provide evidence that the favorable properties of the AA-KO adipocytes secretome may be due in part to the increased adiponectin and decreased IL-6 levels, and to the enhanced MAG levels which activates PPARs. The ABHD6 interactome consists in part of proteins involved in insulin signaling and inflammatory pathways. ABHD6 suppression in preadipocytes inhibits their proliferation and promotes adipocyte differentiation, synergistically with insulin. In insulin resistant female subjects, ABHD6 expression is elevated in the visceral fat and positively correlates with obesity and altered metabolic indices. Overall, the results indicate that adipose ABHD6 suppression prevents the metabolic and inflammation complications of obesity, but not obesity *per se*, possibly via MAG/PPAR activation and enhanced insulin signaling, and that AA-KO mice show promise as a model for the study of MHO.

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Targeting Cysteine Residues as modulators of STAT1 Activity: Implications for Infectious and Autoimmune Disorders

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Background: IFNγ is a prominent proinflammatory cytokine that holds a pivotal position in both inflammation and autoimmune disorders. STAT1, the central transcription factor to the IFNγ response is activated by JAK kinases to mediate the activation of Interferon Stimulated Genes. Dysregulation or mutation of STAT1 results in severe conditions, compromising the body's defense against pathogens or triggering autoimmune responses. This is underscored by Single Nucleotide Polymorphisms (SNPs) of *STAT1* gene. Amongst them, SNPs affecting specific Cysteines cause Gain of Function (GOF) phenotype that has been identified as the etiology of Chronic Mucocutaneous Candidiasis Disease (CMCD) rare disease. This suggests a crucial role of Cys in enabling an effective STAT1-dependent response. Regulation of STAT1 activity through different post-translational modifications is well-characterized. Our study focuses on understanding if and how Cysteines reversible oxidative post-translational modifications (ox-PTMs) contribute to the fine-tuning of STAT1 regulation in the context of IFNγ stimulation. Methods and results: Using maleimide-derivative bioswitch methods to label Cys ox-PTMs and immunoblotting, we demonstrated that STAT1 is subjected to reversible Cys ox-PTMs. Additionally, using the DCP-Bio1 probe, we demonstrate that STAT1 undergoes Cys sulfenylation in response to IFNγ. Analysis of Cys/Ala mutations revealed that mutations in two functional domains are associated with GOF phenotypes, characterized by increased activating phosphorylation, nuclear accumulation, decreased dephosphorylation. RNASeq analysis confirmed increased IFNγ-induced gene expression by the C/A mutants. Moreover, oxidant treatment impaired IFNγ-induced STAT1 phosphorylation. Conclusion: Our data support a model in which previously unrecognized reversible ox-PTMs of STAT1 Cysteine residues dampen STAT1 activity in part through the promotion of STAT1 dephosphorylation. This opens doors for the future development of drugs targeting Cys residues to control STAT1 activity.

Targeting RIPK3-mediated epithelial cell necroptosis protects against RSV infection

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Respiratory syncytial virus (RSV) is the leading cause of hospitalization due to pediatric viral respiratory tract infection, responsible for 200,000 infant deaths worldwide. There are no effective antiviral therapies or clinically approved RSV vaccines for infants. Therefore, understanding RSV-host interactions is crucial for developing new therapies. We have previously shown that RSV infection induces alveolar macrophage necroptosis through the activation of receptor-interacting protein kinase 3 (RIPK3), enhancing disease pathogenesis. Pharmacological inhibition or genetic deficiency of RIPK3 decreases RSV viral load and lung inflammation in mice. Dabrafenib is an FDA- and Health Canada-approved anticancer drug that selectively inhibits RIPK3 as an off-target effect, and lessens tissue injury in different disease models. Thus, we hypothesized that dabrafenib has antiviral effects against RSV by inhibiting RIPK3-mediated necroptosis. We assessed dabrafenib drug repurposing to treat RSV infection *in vitro*. To analyze the effect of dabrafenib on A549 cell viability, cells were treated with increasing concentrations of dabrafenib for different time points using MTT assay. Lactate dehydrogenase (LDH) release was measured as a marker of lytic cell death. A549 cells were infected with RSV-GFP and treated with dabrafenib either simultaneously, prophylactically, or therapeutically. Infection rate and fluorescence intensity were quantified by immunofluorescence. To understand the effect of dabrafenib on viral progeny release, the supernatant of infected A549 cells therapeutically treated with dabrafenib was used to perform a lysis plate titration assay in HEP-2 cells. We also tested the antiviral effects of dabrafenib using human primary nasal epithelial cells (HNECs) and quantified RSV replication by both immunofluorescence and qPCR. Proteomic analyses of epithelial cells infected with RSV and treated with dabrafenib was conducted using quantitative mass spectrometry. Dabrafenib did not alter A549 cell viability throughout 72h at all concentrations tested. Dabrafenib protected A549 cells from RSV-induced lytic cell death. Importantly, the prophylactic, therapeutic, and simultaneous treatments significantly decreased RSV infection rate and fluorescence intensity. Furthermore, therapeutic treatment with dabrafenib significantly reduced the release of RSV infectious progeny. Dabrafenib also reduced in 80% RSV viral load in HNECs. Dabrafenib profoundly altered the A549 cell proteome, inducing the upregulation of some proteins involved in antiviral response. Dabrafenib treatment significantly impairs RSV replication and protects respiratory epithelial cells from death. It also modulates the epithelial cell proteome by upregulating antiviral proteins. Repurposing dabrafenib may be a valuable therapeutic option against RSV infection.

The anti-atherosclerotic agent MPE-298 causes alternate trafficking of the cluster of differentiation 36 receptor (CD36) in macrophages

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Oxidized low density lipoproteins (oxLDL) interact with the cluster of differentiation 36 receptor (CD36), a scavenger membrane protein, at the surface of macrophages. Internalization of oxLDL by CD36 is implicated in initiation of atherosclerosis pathology. The synthetic cyclic azapeptide MPE-298 binds selectively to CD36 with high affinity and exhibits potent anti-atherosclerotic effects in mice. Aiming to decipher the anti-atherosclerotic mechanism of MPE-298, CD36 internalization and intracellular trafficking was investigated in murine macrophages. After transfection with mouse CD36 conjugated to green fluorescent protein (GFP), J774 macrophages were incubated with either MPE-298 (100 nM) or oxLDL (25 μg/mL) for 5 to 15 minutes. The intracellular trafficking of MPE-298 was also monitored in RAW 264.7 macrophages, which were incubated with MPE-298 tagged with ATTO-465 as a fluorescent probe (5 μM) for 10 to 30 minutes. Colocalization with CD36 was examined using specific antibodies for labeling in different endocytic compartments: early (EEA-1), late (Rab7) and recycling (Rab11) endosomes, as well as lysosomes (LAMP-1). Analysis using the ImageJ software indicated that upon exposure to MPE-298, CD36 is rapidly internalized in the macrophage and colocalized within the early and late endosomes and lysosomes, but not within the recycling endosomes. Similarly, the fluorescent MPE-298 analog is also internalized and colocalized within the late endosomes and lysosomes. In contrast, upon macrophage exposure to oxLDL, CD36 colocalizes mainly within the early endosomes. In conclusion, binding of MPE-298 by CD36 at the macrophage membrane induces rapid endocytosis of the CD36-MPE-298 complex and rapid transfer to lysosomal compartments. In addition to negatively modulating CD36 expression on membranes, MPE-298, compared to oxLDL, causes alternate intracellular localization of CD36 within the macrophages. The latter may explain in part the first steps in the anti-atherosclerotic mechanism of MPE-298.

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The BiST of Burden: Harnessing biased STING agonists to enhance the resolution of inflammation and limit tissue fibrosis

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Stimulator of IFN Genes (STING) is a cytosolic DNA sensor that plays a central role in host protection against pathogens upon binding of DNA-derived ligands. STING primarily acts by controlling the transcription of type I interferons (IFNs) and pro-inflammatory cytokines. Notably, STING can be inhibited or activated pharmacologically to control STING-associated pathologies. 5, 6-Dimethylxanthenone-4-acetic Acid (DMXAA) is a pharmacological activator of murine STING that induces IFN-β and its affected genes. Here, we report that macrophages from DMXAA-treated mice engulfed significantly higher numbers of apoptotic cells ex vivo, and exhibited enhanced reprogramming reflected by an increased IL-10 and reduced inflammatory cytokine secretion upon LPS exposure. Macrophage reprogramming was significantly hampered in STING and IFN-β -deficient macrophages. Furthermore, we used virtual docking and batch screening to identify biased STING agonists (BiSTs) that enhanced IL-10 and IFN-β production by splenocytes while inhibiting TNFα. One of these compounds, termed BiST 2.1, also induced the murine STING pathway in vivo and in human macrophages. Finally, we found BiST 2.1 to enhance the resolution of liver fibrosis induced by CCl_4. Thus, our findings indicate that STING can be harnessed to drive IFN-β-mediated IL-10 secretion by resolution phase macrophages and consequently shape macrophage function to enhance the resolution of inflammation and treat fibrotic disorders.

Therapeutic strategies to mitigate diastolic dysfunction in arthritis: targeting IL-6 and Galectin-3

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Rheumatoid arthritis (RA) patients have double incidence of heart failure with preserved ejection fraction (HFpEF), a risk that may augment with current treatments, including glucorticoids and anti-TNF therapies. New models and targets are required to address this unmet clinical need [PMID: 36041475]. The KBxN F1 mouse colony develops arthritis prior to develop diastolic dysfunction: these animals respond to the pro-resolving therapeutic with human recombinant Annexin A1 [PMID: 34526398]. Herein, we targeted IL-6 and Galectin-3 (Gal-3), since both these mediators were elevated in KBxN F1 mice prior to cardiac alterations. Treatment of male and female mice (n=10 per group) with anti-IL-6 receptor antibody (MR16-1) from week 6 to week 12, significantly i) prevented the increase of the left atrium area (LAA) by ~10% and ii) improved the E/A ratio and E'/A' wave ratio ($p<0.05$ in both cases). Ejection fraction did not vary among the experimental groups. MR16-1 reduced arthritic score and paw oedema by ~15% as compared to the vehicle group. Functional cardiac changes were associated with lower recruitment of $Ly6C^{hi}$ $CRR2^{+}$ monocytes to the heart at week 12, together with lower pro-inflammatory fibroblast numbers ($p<0.05$). Finally, treatment of mice with MR16-1 significantly reduced the expression of Gal-3+ in cardiac macrophages (MHCII+). Since Gal-3 is a pro-fibrotic protein which circulating levels increase in KBxN F1 mouse plasma over the disease timeline, the effect of a Gal-3 inhibitor (GB1107) was then tested. Daily administration of GB1107 (10 mg/kg orally; week 6-12), did not affect the arthritic score nor paw oedema. Similarly to MR16-1, the Gal-3 inhibitor GB1107 reduced LAA enlargement by ~12% and improved the E/A ratio and E'/A' ratio. In summary, KBxN F1 transgenic mice develop diastolic dysfunction and this experimental model can be used to elucidate mechanistic alterations in the heart as well as allow testing of effecrtive therapeutic approaches. Our ultimate goal is to prevent development of HFpEF in RA patients, a pathology markedly ignored by current treatment regimens.

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Thrombopoietin-dependent megakaryopoiesis fuels thromboinflammation and worsens antibody-mediated chronic renal microvascular injury

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Background. Small vessel vasculitides (SVVs) are rare, autoimmune, and life-threatening diseases affecting many organs, including the kidney. SVVs trigger chronic thromboinflammation in the renal microvascular network. This process provokes microvascular alterations and rarefaction, promoting renal dysfunction and the development of glomerulosclerosis, all of which are responsible for the development of end stage renal disease (ESRD). We hypothesized that hematopoietic growth factors (HGFs) released by the inflamed kidney may sustain "emergency hematopoiesis" and fuel the thromboinflammatory process.

Method. Using a murine model of antibody-mediated chronic kidney disease (AMCKD), mimicking the human pathology, and pharmacological interventions, we comprehensively monitored the response to injury in the circulating blood, urine, bone marrow, and kidney.

Results. Experimental AMCKD is characterized by chronic thromboinflammation in glomeruli and the production of HGFs, especially thrombopoietin (TPO), by the injured kidney, which stimulated and skewed hematopoiesis toward megakaryopoiesis. AMCKD was characterized by vascular and kidney dysfunction, TGFβ-dependent glomerulosclerosis and microvascular rarefaction. In humans, chronic thromboinflammation in glomeruli during SVV-induced glomerulonephritis is associated with TGFβ-dependent glomerulosclerosis and increased bioavailability of TPO, amongst other HGFs and pro-inflammatory cytokines. The combined analysis of albumin, TPO and pro-inflammatory cytokine levels in sera from patients with SVV-induced glomerulonephritis allowed us to identify treatment responders. Strikingly, TPO neutralization in the experimental AMCKD model normalized hematopoiesis, reduced chronic thromboinflammation, vascular and kidney dysfunction, TGFβ-dependent glomerulosclerosis and microvascular rarefaction.

Conclusion. Our results show that TPO-skewed hematopoiesis exacerbates chronic thromboinflammation in microvessels and worsens AMCKD. TPO is both a relevant biomarker and a promising therapeutic target in humans with CKD and other chronic thrombo-inflammatory diseases.

Transforming Growth Factor Beta (TGFβ) Reduces Neutrophil Motility by Promoting Formation of Neutrophil Clusters in Lung Capillaries

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Major trauma is any tissue injury that can lead to disability or death. Regardless of the site of trauma, patients suffer from acute lung failure and often require ventilation. Unfortunately, ventilator-dependence increased the risk of bacterial lung pneumonia. Using a mouse trauma model, in the form of skin burn injury, we revealed that the infiltration of granulocytes in the lungs, praticularly neutrophils, contribute to a loss of pulmonary function. Early post-injury, systemically upregulated transforming growth factor beta (TGFβ) drove neutrophil accumulation and clustering (cell-cell interactions) in the lung capillaries, and elevated expression of program death ligand-1 (PD-L1) and cluster of differentiation 80 (CD80) on neutrophils. However, the function of PD-L1 and CD80 on neutrophils is unclear. Neutrophils are known to be highly motile, allowing them to respond swiftly to infection. The mechanism by which injury-induced TGFβ affects neutrophil microbicidal ability, however, remains unkown.

We hypothesize that neutrophils cluster through PD-L1 and CD80 interaction, resulting in reduced motility and compromised microbicidal ability.

Using extracted mouse neutrophils, confocal microscopy revealed colocalization of PD-L1 and CD80 on clustered neutrophils following TGFβ treatment. To determine how TGFβ-induced clustering affects neutrophil motility, we generated mice with neutrophil-specific TGFβ receptor 2 (Tgfbr2) depletion ($Tgfbr2^{fl/fl}$ $MRP8^{cre/+}$). Lung intravital microscopy revealed that, while injury reduced displacement and velocity of WT mice neutrophils, $Tgfbr2^{KO}$ neutrophils partially restored their migration ability.

Burn injured patients are acutely susceptible to bacterial pneumonia with no alternative treatment for multi-drug resistant pathogens. In this study, we revealved a TGFβ induced PD-L1-CD80 inetraction on neutrophils, causing clustering and restricted motility. Characterization of injury-induced phenotypic and functional changes of neutrophils insight into alternative treatments for antimicrobial bacterial pneumonia in not only burn-injured patients, but also other major trauma and infectious diseases.

Translating Pharmacokinetic and Efficacy Outcomes of NLRX1 Agonist NX-13: Contrasting a Pig Model and a Human Phase 1b Clinical Trial In Ulcerative Colitis

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In rodent models of ulcerative colitis (UC), NX-13 (an orally active, gut selective NLRX1 agonist) improves disease activity through a novel bimodal mechanism. A pig DSS colitis study was performed to generate pharmacokinetic (PK) and mechanistic data, given pigs can be dosed with tablets and their gut and immune systems are markedly more similar to humans. We describe the efficacy and PK of NX-13 tablets in pigs compared to phase 1 clinical trials. NX-13 immediate release (IR) tablets were used in pig studies and human clinical trials with plasma samples collected at hourly intervals. Stool samples were collected at ~24hrs after last dose. The phase 1 clinical trials were double blind, randomized placebo-controlled studies in healthy subjects (1a, Leber et al *UEGW J* 9(Supp)) or subjects with active UC (1b, Verstockt et al *JCC* epub 11NOV2023). NX-13 concentrations were determined by LC-MS/MS. In pigs, oral NX-13 dampened colitis as early as day 2, becoming significant at days 4-6 (Fig. 1A). Consistent results were observed in the Phase 1b trial, including rectal bleeding improvement as early as week 2 (Fig. 1B) and endoscopic improvement at week 4. Despite signs of sufficient target engagement in both species, the NX-13 PK characteristics differed. Firstly, NX-13's low level of human systemic exposure peaked 1 hr after dosing, whereas Tmax in pigs was 7 hrs at comparable doses. Secondly, the NX-13 retained in the stool is greater in pigs (250-1000x plasma levels), compared to humans (150-400x) although both stool and plasma concentrations were greater in pigs (Fig. 1C). This suggests NX-13 is not gut-restricted in humans, but rather gut-selective with low systemic absorption. Interestingly, the stool:plasma ratio was numerically lower in patients with UC, implicating epithelial barrier integrity. The NX-13 PK hypothesis of a gut-selectivity with low systemic absorption will be analyzed in the ongoing phase 2 trial.

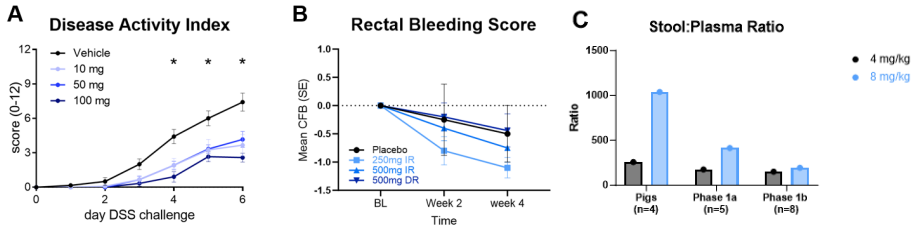

Figure 1: NX-13 effectively dampens colitis severity in a DSS colitis model in pigs (A) while similarly inducing symptomatic improvement in RBS in a phase 1b clinical trial (B). Ratios of NX-13 in stool to NX-13 systemically absorbed in plasma was greater in pigs (C).

Unlocking the Pharmaceutical Potential of Specialized Pro-resolving Mediators by Chemical Synthesis of Simplified Structural Mimics

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Specialized pro-resolving mediators (SPMs) including protectins, resolvins and maresins are recognized as important players in resolution of inflammation, and their therapeutic potential has been highlighted in many different models of inflammation related diseases. However, their translation into potent pharmaceutical drugs still encounters important hurdles including prohibitive cost of production, long chemical synthesis routes, patentability as natural product class, chemical sensitivity/instability, and sub-optimal physicochemical properties. In order to favor their emergence as valuable pharmaceutical class of compounds, we designed a novel strategy to simplify their chemical syntheses as well as to increase their related molecular diversity. Indeed, we have developed a molecular platform of simplified mimics of SPMs that structurally conserved the configuration of the central allylic trienic system (either E,Z,E; Z,E,E and E,E,E), but in varying their side chains. Protectin analogues with variable substituent groups were synthesized in 10-12 chemical steps to illustrate the viability of the approach. Interestingly, among the 40 protectin analogs synthesized, higher anti-inflammatory activities than their corresponding natural protectins (protectin DX and D1) were obtained in lipopolysaccharide (LPS) stimulated J744 macrophages, as well as enhanced antiviral activities on influenza and Sars-Cov-2 viruses. Some of these protectins analogues also showed promising preliminary pharmacokinetic profiles. These initial results reveal the potential of such structurally simplified mimics of SPMs as a valuable strategy towards their translation as future drug candidates.

Unlocking the Potential of Omega-3 Fatty Acids: Modulation of Vascular Tone in Pulmonary Hypertension

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Pulmonary hypertension (PH) is a severe disease that arises from multiple etiologies, leading to right ventricular failure and death. Pulmonary perivascular inflammation has gradually gained increased attention as an early common hallmark across different PH groups. Most of the used treatments target the pulmonary vasoconstriction (PGI_2 analogus, ET-1 inhibitors and Phosphodiesterase inhibitors) that stimulate smooth muscle cell relaxation. However, pulmonary hypertension remains associated with significant morbidity.We therefore hypothesised that inflammation plays a crucial role in the severity of abnormal vasoconstriction in PH. Based on this hypothesis, we have selected a candidate family of bioactive lipids: omega-3 fatty acid (EPA, DHA and DPA) and their metabolits with high resolving potential, called the SPM for specialised proresolving mediators (resolvins, protectins and maresins).

Human pulmonary arteries (HPA) derived from PH or non-PH patients were gathered at Bichat hospital. Using an isolated organ system, we have assessed the functional effects of EPA, DHA, and DPA alone and in combination with vasoactive compounds relevant to the pathology. Furthermore, we have examined the underlying mechanisms of the observed effects on each signalling pathway by using western blot and ELISA. We measured the endogenous SPM expressed in pulmonary vascular tissue (+/- PH) with LC/MS-MS after a stimulation with omega-3, and finally we describe the localization and transcript level of the enzymes involved in the SPM biosynthesis and their receptors in the human pulmonary vascular wall respectively with RT-qPCR and immunofluorescence.

In summary, our findings indicate that omega-3 fatty acids, and their metabolites the SPM possess inherent vasorelaxant properties, especially in non-PH HPA. However, in the context of PH, these relaxing effects seem to diminish. Additionally, our investigations unveiled intriguing interactions between omega-3 fatty acids, notably DHA and DPA, with HPA responses to Iloprost (enhancement of vasorelaxation) and Prostaglandin E_2 (reduction of vasoconstriction) for both non-PH and PH HPA.

Visceral Pain in Inflammatory Bowel Disease: a role for Protease-Activated Receptor-1

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Pain is a cardinal sign of inflammation and is associated with flares in inflammatory bowel disease (IBD) patients, where peripheral mediators released by inflamed tissues are responsible for activation of nociceptors and pain pathways. However, 30 to 50% of IBD patients in remission still report significant pain, despite the absence of infiltrated inflammatory cells and complete repair of tissues. We hypothesized that peripheral (colonic) mediators present in repaired mucosa can signal to sensory neurons contributing to pain symptoms. We have investigated the effects of colon biopsy supernatants from IBD patients in remission or healthy controls on cultured dorsal root ganglia (DRG) neurons harvested from mice or human cadavers and tested the effects of Protease-Activated Receptor-1 (PAR1) antagonist. Further, we have induced colitis in rats, by the intracolonic administration of trinitrobenzene sulfonic acid (TNBS), and recorded nociceptive responses. Colon biopsy supernatants from IBD patients in remission but not from healthy controls caused significant activation (calcium signal) of DRG neurons both in mouse and human neurons. Pre-incubation of neurons with a PAR1 antagonist significantly inhibited IBD supernatants-induced DRG calcium signals. Oral treatment with the PAR1 antagonist CVT120165 significantly inhibited, dose-dependently, referred abdominal pain in rats induced by intracolonic TNBS administration 7-days earlier. PAR1 antagonist also favored tissue repair. Peripheral mediators in the colon of IBD patients in remission are able to activate sensory neurons by a PAR1-dependent mechanism. PAR1 blockade relief from pain symptoms in a rat model of colitis, suggesting that PAR1 could be a good target for the treatment of IBD-associated pain in remission.

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Abstract Book (Posters)

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A Comparison of the IVIV Translation of a Degrader with an Inhibitor of IRAK4

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Interleukin-1 Receptor-Associated Kinase 4 (IRAK4) is a master regulator of innate immunity, playing a central role in both toll-like receptor (TLR) and interleukin-1 (IL-1β) inflammation. IRAK4 regulates cytokine transcription important to the autoimmune pathophysiology, including atopic dermatitis (IL-1α/IL-1β) and asthma (IL-33). Proteolysis targeting chimeras (PROTACs) are hetero-bifunctional molecules that degrade target proteins using the ubiquitin–proteasome system. PROTACs are a novel therapeutic strategy to target IRAK4, which has the efficacy advantage of removing both the scaffolding and kinase function of the protein compared to small molecule kinase activity inhibitors like PF-06650833/Zimlovisertib, which was discontinued in Phase 2. Recently, an IRAK4 PROTAC, KT-474, has entered Phase 2 clinical trials for the treatment of hidradenitis suppurativa and atopic dermatitis. This provided an opportunity to compare the *in vitro* to *in vivo* translation of an IRAK4 degrader with an activity inhibitor. Initially, we characterized the ability of KT-474 and PF-06650833 to degrade IRAK4 and inhibit downstream LPS-driven cytokine production using the THP-1 cell line, human peripheral blood monocytes (PBMCs), and mouse splenocytes. Furthermore, we have determined the PK of KT-474 and PF-06650833 prior to profiling both compounds in a mouse LPS model of acute inflammation. Our results demonstrate that KT-474 potently degrades IRAK4 (0.88 nM DC50, 101% Dmax) and inhibits LPS/R848-driven PBMC IL-6 production. Importantly, the inhibitory effect of KT-474 is maintained following its removal, unlike PF-06650833, demonstrating the longevity of this therapeutic modality over kinase activity inhibitors. In mouse PK, KT-474 reaches a Cmax after 2 hours, and there are measurable plasma levels up to 24 hours. This data has been used to design a mouse LPS PD model, in which IRAK4 degradation and inflammatory cytokines suppression will be used to evaluate PK PD relationships. To conclude, we have demonstrated that the degradation of IRAK4 by KT-474 is an effective therapeutic modality to inhibit cytokine generation with potential advantages over conventional kinase activity inhibitors.

A novel point of intervention for mitigating macrophage hyper-inflammation and the cytokine storm in coronavirus disease

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The COVID-19 pandemic due to the coronavirus SARS-CoV-2 has been responsible for nearly 7 million deaths globally since 2020. In spite of vaccines, the virus continues to propagate, with the immunosuppressed, aged, and non-vaccinated populations remaining vulnerable. An unmet therapeutic challenge caused by COVID-19 is acute respiratory distress syndrome (ARDS), which develops typically from pulmonary hyper-inflammatory response degenerating into the so-called cytokine storm. The cluster of differentiation 36 receptor (CD36) is expressed in macrophages and associated with the inflammatory response. Ligands of the CD36 receptor have been shown to reduce inflammation in murine models. Hypothesizing the potential to curb ARDS in COVID-19, the CD36 ligand hexarelin, an analog of the growth hormone-releasing peptide (GHRP) family, was examined for the ability to attenuate hyper-inflammation of pulmonary macrophages. Dose-response studies for determining the optimal dose of hexarelin were performed in a non infectious lung inflammation model. Transgenic mice expressing the human ACE2 protein under regulation of the human cytokeratin 18 promoter in epithelial cells (K18-hACE2) were treated by a subcutaneous injection of hexarelin (10 μmol/kg) or 0.9% NaCl vehicle 30 minutes before intranasal instillation with SARS-CoV-2 (250 TCID50), and then treated daily with hexarelin or vehicle over the next 9 days. Survival and body weight were documented daily. Cytokine levels were assayed in lung homogenates. Hexarelin increased survival, decreased body weight loss and reduced pro-inflammatory pulmonary cytokine and chemokine levels in lung homogenates. Modulation of CD36 offers potential as a novel therapeutic approach for mitigating virus-induced hyper-inflammatory response of lung macrophages in SARS-CoV-2 infection.

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A Peptide Formulation Based on Annexin A1 as a Host-directed Therapy Against Dengue Disease

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Severe dengue (DG) is marked by intense inflammation, causing cytokine storm, resulting in vascular leakage, hemorrhage, and shock. Innate immune cells and products are vital in these processes. No licensed antiviral drugs or host-targeting therapies exist for dengue, underscoring the urgent need for new treatments. Our research has identified Annexin A1 (AnxA1) as a critical mediator of the resolution response in dengue. AnxA1 acts through its FPR2/ALX receptor expressed on various cell types, including leukocytes, mast cells and endothelial cells. We observed reduced circulating levels of AnxA1 in dengue patients, particularly in those with severe disease, as well as in DENV-infected mice. AnxA1 knockout (AnxA1KO) mice displayed increased susceptibility and delayed disease resolution, emphasizing the importance of AnxA1 in dengue pathogenesis. Conversely, treatment of mice with the AnxA1 mimetic peptide (Ac2-26) improved disease outcomes without compromising the host's ability to deal with the infection. However, systemic administration of proteins like AnxA1 is hindered by instability and rapid degradation. To address this, we created a formulation using cyclodextrins (CDX), known for stability and enhanced bioavailability. We utilized Hydroxypropyl-β-cyclodextrin (HP-BCD) to improve the stability and *in vivo* effects of Ac2-26. Experiments were conducted on A129 mice infected with DENV-2, treated with pure or formulated Ac2-26 via intraperitoneal (i.p.) or oral routes of administration, starting 36 hours post-infection. Euthanasia occurred at days 3 or 5 post-infection, representing the peak of viral replication and disease manifestation, respectively. Results showed that both pure and formulated Ac2-26 treatments reversed clinical scores and thrombocytopenia induced by DENV infection. Moreover, formulated Ac2-26 exhibited potentiated anti-inflammatory effects by reducing mast cell degranulation and plasma levels of MCPT-1, as well as, reducing levels of several cytokines in the spleen and liver damage induced by infection. Notably, the formulation containing Ac2-26 did not impact DENV titers, indicating its selective anti-inflammatory and pro-resolving properties. Finally, the association of formulated Ac2-26 with and antiviral drug (a nucleotide analogue), fully prevented mice from DENV-induced disease and lethality.These findings underscore the importance of developing combined therapeutic approaches that aim for synergistic effects, leading to more effective strategies with reduced side effects on the host.

Activation of astrocytic EMMPRIN contributes to neuroinflammation in Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal disease characterised by the degeneration of upper and lower motoneurons. Onset and progression of the disease are determined by both cell-autonomous neuronal dysfunctions and non-cell-autonomous factors, mainly due to activation of glial cells. Activated astrocytes posses both neurotoxic and neuroinflammatory properties in ALS, and the study of the molecular pathways underlying their activation may reveal new therapeutical targets to counter their toxicity.

The Extracellular Matrix Metalloproteinases INducer (EMMPRIN), a glycoprotein expressed by various cell types including neurons, is the major activator of matrix metalloproteinases (MMP) synthesis and release. EMMPRIN activation can be induced by peptidyl-prolyl isomerase A (PPIA), a chaperone protein with cis/trans isomerase activity, that exhibits cytokine- and chemokine-like behaviour. Interestingly, PPIA is highly released in the cerebrospinal fluid (CSF) of ALS patients and animal models where, by activating EMMPRIN on motoneurons, induces MMP-9 expression and neuronal death.

Here we show that EMMPRIN is expressed also by astrocytes where it is upregulated under inflammatory conditions or in presence of the $SOD1^{G93A}$ mutation that characterises familial ALS cases. PPIA-mediated stimulation of EMMPRIN in healthy astrocytes induces NF-kB activation and a proinflammatory phenotype. Interestingly, $SOD1^{G93A}$ astrocytes release high levels of PPIA and present the same activated phenotype induced by PPIA in healthy astrocytes. Finally, treatment of $SOD1^{G93A}$ astrocytes with an anti-EMMPRIN antibody, known to block PPIA/EMMPRIN interaction, reverses the toxic phenotype and suggests an autocrine mode in the astrocytic activation of EMMPRIN.

In conclusion, here we demonstrate that EMMPRIN activation in ALS occurs also in astrocytes where it exacerbates their neuroinflammatory and toxic phenotype. Furthermore, we suggest the potential use of an anti-EMMPRIN antibody to reduce astrocytic activation during the disease.

Alterations of B cells in hypersensitivity pneumonitis and their modulation by $S1P_1$ ligands

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Hypersensitivity pneumonitis involves antigen-induced inflammatory flares, however its etiology remains misunderstood. Consequently, the diagnosis is complex and evidence-based therapies are lacking, especially in the chronic fibrotic stages of the disease. B cell-derived mediators, including pro-inflammatory cytokines and antigen-specific antibodies, are central to the pathogenesis, and experimental models of hypersensitivity pneumonitis support the concept that B cell functions are modulated by the sphingosine-1-phosphate receptor 1. The aims of this exploratory study were to characterize lymphocyte populations in patients with chronic hypersensitivity pneumonitis and to determine if sphingosine-1-phosphate receptor 1 ligands directly interfere with B cell functions. In this study, venous blood of eleven chronic hypersensitivity pneumonitis patients and ten control subjects was processed for flow cytometric/functional cellular analyses and quantification of soluble mediators. Isotype-switched circulating memory B cells were reduced while naïve B cell frequencies were increased in patients compared to control. Contrarily to B cells, T cell subpopulations were similar in both groups. Plasmatic concentration of IL-21, TNF and B cell survival factor BAFF were increased in patients. *Ex vivo*, sphingosine-1-phosphate receptor 1 ligands modified surface expression of activation markers on B cells and the release of TNF and IL-6 in response to a T cell-independent antigen. We conclude that chronic hypersensitivity pneumonitis leads to changes in circulating B cell subpopulations and that sphingosine-1-phosphate receptor 1 modulators interfere *ex vivo* with the activation of circulating B cells in response to a T cell-independent antigen. The impact of sphingosine-1-phosphate receptor 1 ligands on T cell activation after CD3/CD28 stimulation is currently under investigation.

Alzheimer's disease microglia exhibit an enrichment of somatic cancer driver mutations, correlating with disease-associated inflammatory states

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Alzheimer's disease (AD), an age-associated neurodegenerative disorder, is characterized by progressive neuronal loss and the accumulation of misfolded proteins like amyloid-β and tau. Neuroinflammation, mediated by microglia and brain-resident macrophages, plays a pivotal role in AD pathogenesis, yet the intricate interactions among age, genes, and other risk factors remain elusive. Somatic mutations, known to accumulate with age, instigate clonal expansion across diverse cell types, impacting both cancer and non-cancerous conditions. Utilizing molecular-barcoded deep panel sequencing on 311 prefrontal cortex samples of AD patients and matched controls, our study unveiled an elevated occurrence of somatic mutations within cancer driver genes in AD brains. Recurrent somatic mutations, often multiple, were observed in genes associated with clonal hematopoiesis (CH). Remarkably, these somatic cancer driver mutations were specifically enriched in CSF1R+ microglia of AD brains and exhibited signs of positive selection, suggesting mutation-driven microglial clonal expansion (MiCE). Single-nucleus RNA sequencing of the temporal neocortex samples from additional 62 AD patients and matched controls revealed a nominal increase in subchromosome-level somatic mutations associated with CH in AD microglia, with mutant microglia exhibiting upregulated pro-inflammatory and AD-associated genes. We further created three lines of induced pluripotent stem cell (iPSC)-derived microglia, each carrying driver mutations in one of the most mutated genes in AD brain. Through single-cell RNA sequencing, we verified that these mutant microglia consistently displayed an amplified inflammatory and disease-associated transcriptional profile compared to their wild-type counterparts. Our findings indicate that somatic cancer driver mutations in microglia are prevalent in normal aging but further enriched in AD, driving MiCE and promoting inflammatory, disease-related microglial signatures. This study provides crucial insights into microglial clonal dynamics in AD, potentially paving the way for novel approaches to AD diagnosis and therapy.

Analysis of the contribution of mutations in tumor suppressor genes in the pathogenesis of ovarian cancer in Crimean patients

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Ovarian cancer is aggressive tumor characterized by one of the highest mortality rates among oncogynecological cancers. The most crucial factor in ovarian cancer development is genetic disturbances including mutations leading to tumor suppressor genes inactivation. There are a lot of various genes that take part in maintaining cell DNA stability but *BRCA1* and *BRCA2* are the most known in context of varian cancer pathogenesis. Moreover, ovarian cancer is characterized by high genetic heterogeneity. The frequency and spectrum of mutations inducing cancer development is variable depending on geographical and ethnic affiliation.

This paper presents the analysis of the frequency and spectrum of mutations in *BRCA1, BRCA2, CHEK2, PALB2* genes and their role in the development of ovarian cancer in Crimea.

The study involved 100 Crimean residents of various ethnic groups. 60 women with ovarian cancer were sorted into the study group. Control group was formed with rest healthy women without oncological diseases family history

Mutations in the *BRCA1* gene were found in 5 in the study group, which amounted to 12.0%. The mutation spectrum included C.5382insC (2.4%), C.300T>G (2.4%), C.185delAG (2.4%), C.4153delA (4.8%). *BRCA1* mutations weren't detected in the control group. There're were two *CHEK2* gene mutation C.470T>C carriers was found in among study group (4.8%) and four carriers in the control group (9.6%). One of the women with the mutation from the control group belongs to the Tatar ethnic group and it is important to hightlight that this s rare case.. Mutations in the *BRCA2* and *PALB2* genes were not detected.

Germinal mutations in the *BRCA1* gene dominated among ovarian cancer patients, however, in total, the *CHEK2*c.470T>C mutation is characterized by the highest frequency of occurrence in both studied groups. To date, discussions are underway about the contribution of this pathological variant to the development of malignant neoplasms of various localization, however, this mutation should be included in diagnostic panels in the Crimea.

Annexin A1 in the Pathogenesis of Atopic Dermatitis: Effect on Keratinocytes

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Annexin A1 (AnxA1) is a glucocorticoid-induced protein with potent anti-inflammatory by regulation of inflammatory mediators and activation of NLRP3 inflammasome. Considering that the role of AnxA1 in normal and inflamed skin is not completely defined, this study evaluated AnxA1 and NLRP3 levels in skin biopsies from control and atopic dermatitis (AD) patients, and examined their effects on human keratinocytes stimulated with IL-4. Two studies containing publicly available transcriptome data (GSE16161 and GSE1120721) were individually analyzed using the GEO2R tool to detect AnxA1 and NLRP3 mRNA levels. For protein detection, paraffin-embedded human skin biopsies from AD patients and controls (n=10/group) were processed for immunohistochemical analyses. *In vitro*, IL-4-stimulated keratinocytes, HaCaT lineage, were treated with or without AnxA1-derived peptide Ac_{2-26} (5 or 25 ng/mL); some cells received 15 min before Ac_{2-26}, the pan-formyl peptide receptor (FPR) antagonist Boc2 (10 μM). Transcriptome analyses of GSE16161 showed a significant increase in *ANXA1* and *NLRP3* transcripts in AD skins compared to the control skins, while in study GSE120721 only *NLRP3* transcripts showed a significant increase. In study GSE120721, separate evaluation of the epidermal transcriptome showed elevated levels of *ANXA1* transcripts in AD lesional skin relative to non-lesional AD skin, while transcriptional levels of *NLRP3* were reduced in the lesional AD epidermis compared to controls. Immunohistochemical analysis of AD skin biopsies showed coexpression of AnxA1 and NLRP3 in the cytoplasm of keratinocytes. Under IL-4 stimulation, Ac_{2-26} at 25ng produced a marked decrease of keratinocyte proliferation rate, effect abrogated by Boc2. This highest concentration of Ac_{2-26} also decreased NLRP3 levels, IL-1β release and ROS production by IL-4-stimulated keratinocytes in comparison to non-treated cells. Altogether, these findings suggest that AnxA1 exerts an immunomodulatory effect on keratinocytes and contributes to epidermal homeostasis through regulation of cell proliferation provoked by AD-induced inflammatory microenvironment. Funding: FAPESP, CAPES, CNPq.

Annexin-A1-Derived Peptide Ac_{2-26} Presents Neuroprotective and Anti-Inflammatory Effects in Murine Model of Parkinson's Disease

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Annexin A1 (AnxA1) is a calcium-dependent phospholipid-binding protein that plays an important role in regulating neuroinflammation and innate immunity. However, the role of AnxA1 in Parkinson's Disease (PD) was poorly explored. This study presents the role of AnxA1 in a PD mouse model induced by 6-hydroxydopamine (6-OHDA). Male C57BL/6 $AnxA1^{+/+}$ and $AnxA1^{-/-}$ mice were distributed in two groups: PD+Saline and PD+Ac_{2-26}. Both groups underwent stereotactic surgery for intracerebral injection of 1 µL of neurotoxin 6-OHDA (5 µg/µl) directly into right striatum (ipsilateral) and 1 µL of vehicle on the left (control; contralateral). The PD+Ac_{2-26} group was treated with Ac_{2-26} (100 µg/animal) intraperitoneally after the 6-OHDA infusion for 7 days; PD+Saline received only saline. Cylinder test was performed to assess sensory-motor function of mice on 0-, 7- and 14-days post-lesion. All saline-treated mice showed significant decreased contralateral forelimb use compared to ipsilateral on days 7 and 14. Ac_{2-26} treatment produced a reversal of forelimb use asymmetry for both genotypes and no differences were detected between use contralateral and ipsilateral on days 7 and 14. Western blotting corroborated these findings. A significant reduction in tyrosine hydroxylase (TH) levels was detected in the right striatum and substantia nigra (SN) from both genotypes compared to the left side (control). Ac_{2-26} administration reversed this effect and no differences were detected for TH levels in right and left sides of the striatum and SN. Lack of endogenous AnxA1 was associated with reduced levels of the IL-4 on both sides analyzed (R, L) of striatum and SN, regardless of systemic treatment (saline or Ac_{2-26}). In $AnxA1^{-/-}$ SN, the addition of Ac_{2-26} produces a broad anti-inflammatory effect with a reduction in the levels of IL-1β, IL-6, IL-17 and TNF-α, especially on the right side (6-OHDA), compared to $AnxA1^{+/+}$ SN. Taken together, our results show that in the PD model, the endogenous lack of AnxA1 does not worsen the development of the disease. On the other hand, Ac_{2-26} therapy shows neuroprotective and anti-inflammatory effects on SN in both genotypes, highlighting its potential use in neurodegenerative diseases. Funding: FAPESP, CAPES, CNPq.

Associations between vitamin D status and biomarkers linked with inflammation in patients with asthma: A systematic review and meta-analysis of interventional and observational studies

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Abstract

Background: Numerous studies indicate an association between vitamin D status and inflammatory biomarkers in patients with asthma, but findings are inconsistent. This review aims to summarize the relationship between serum vitamin D status, assessed by 25-hydroxyvitamin D (25(OH)D) level, and inflammatory biomarkers.

Methods: A literature search of interventional and observational studies on 25(OH)D up to November 2022 was conducted across six electronic databases. The outcomes of interest included a range of inflammatory biomarkers classified in four distinct categories (T helper 2 (Th2) pro-inflammatory biomarkers, non-Th2 pro-inflammatory biomarkers, anti-inflammatory biomarkers, and non-specific biomarkers). The characteristics and the risk of bias of the studies were extracted and evaluated by independent reviewers. Meta-analysis was conducted on studies with a low risk of bias, while narrative reporting was used to present the direction of associations (positive, no association, or negative) for each biomarker, within the subset of low-risk studies and across all included studies.

Results: We included 71 studies (3 interventional, 68 observational) investigating the association between vitamin D status and Th2 pro-inflammatory biomarkers (N=58), non-Th2 pro-inflammatory biomarkers (N=18), anti-inflammatory biomarkers (N=16), and non-specific biomarkers (N=10). Thirteen (18.3%) studies, 50 (70.4%) and only 8 (11.3%) were at high, neutral, and low risk of bias, respectively. Only one meta-analysis could be performed. The pooled estimate for 25(OH)D and serum IgE showed a negative association (β (95% CI) = –0.33 (–0.65 to –0.01); I^2 = 88%; $P < 0.01$; N=4 studies). In studies at low risk of bias reporting statistically significant results, there was a negative correlation between vitamin D status and both serum IgE and blood eosinophil, whereas a positive correlation with LL-37 was observed. When considering all studies regardless of bias risk, those with statistically significant results predominantly indicated a negative association of 25(OH)D with pro-inflammatory biomarkers and a positive association with anti-inflammatory biomarkers. Nevertheless, the majority of studies reported non-statistically significant relationships with 25(OH)D.

Conclusion: Serum 25(OH)D is negatively associated with serum IgE. Whereas incomplete reporting and non-adjustment for confounders prevented a meta-analysis of other biomarkers, most studies with statistically significant results support a potential anti-inflammatory effect of 25(OH)D.

Balsacone C and Phloretin, Anti-inflammatory and Antiproliferative Polyphenols; Their Assessment in a Psoriatic Model of T cells and Psoriatic Keratinocytes

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Psoriasis is an inflammatory skin disease characterized by thick red plaques and by important leucocyte infiltration, especially of T lymphocytes and dendritic cells. Lack of effectiveness and toxic side effects are the main concerns with conventional treatments, and research involving new antipsoriatic molecules is essential. Recently, the anti-inflammatory properties of polyphenols have attracted interest. A systemic administration of balsacone C has already induced improvement in a 3D psoriatic skin model; epidermal thickness and cell differentiation were more comparable to healthy reconstructed skin after the treatment. However, its anti-inflammatory properties have never yet been evaluated in a psoriatic model. Additionally, the anti-inflammatory activity of phloretin has been reported in the literature, but the compound has never been used on psoriatic keratinocytes to examine inflammation and cell proliferation. In this study, the main objective was to determine the anti-inflammatory and antiproliferative effects of two polyphenols, balsacone C and phloretin, in a coculture of T cells and psoriatic keratinocytes. The treatments were administrated for one week at their median inhibitory concentrations (125 μM balsacone C and 166 μM phloretin) and compared with methotrexate, a reference treatment for psoriasis. The coculture of psoriatic keratinocytes and T cells (N = 3 psoriatic keratinocyte donors, n = 3 cocultures/condition) were compared with healthy keratinocyte cultures (N = 3 healthy donors, n = 3 cultures). Phloretin and balsacone C reduced cell proliferation; the expression of Ki67 and PCNA was lower with phloretin and comparable to the healthy control, and balsacone C reduced Ki67 expression. Although the expression of IL-1α and IL-1β was reduced with balsacone C, phloretin reduced the expression of many more cytokines, including TNF-α, IL-17A, CCL2, MIP-1α, G-CSF, GM-CSF, IL-1α, IL-1β and IL-6, and also increased IL-2 secretion. The level of expression of CD45, a protein expressed on T cells, was closer to that of the healthy control after using phloretin.

Berry e-cigarette vapour impair alveolar macrophage mobility and infection clearance

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In our lower respiratory tract, the alveolar spaces are devided from the bloodstream and the external environment by only a few microns of interstitial tissue. Alveolar macrophages (AMs) defend this delicate mucosal surface from invading infections by regularly patrolling the site. Using intravital microscopy we have uncovered that AMs display three behaviour modalities to achieve the goal of proper survelience of the alveolar space: (i) extending cell protrusions to sample surrounding areas, (ii) squeezing their whole cell body between alveoli, and (iii) patrolling by moving their cell body around each alveolus. In this study we found Rho GTPase, cell division control protein 42 (Cdc42) expression significantly decreased specifically after berry-flavoured e-cigarette (e-cig) exposure. This resulted in a shift in AM behaviour from squeezing to probing. Alterations is AM behaviour resulted in

decreased clearance of inhaled bacteria, *Pseudomonas aeruginosa*. Together these data unravel important pathways in AM migration and show that flavored e-cig vapes have deleterious effects on immune cell function.

Biosynthesis of 5-HETE-Glycerol and 5-KETE-Glycerol, Two New Lipid Mediators Found In Vivo and Potentially Involved in Inflammation

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INTRODUCTION. Granulocyte infiltration is a fundamental feature of inflammation. In tissues, they produce/release soluble mediators including eicosanoids derived from 5-lipoxygenase (5-HETE, leukotrienes). They also biosynthesize the endocannabinoid 2-arachidonoyl-glycerol (2-AG) using a pathway involving the acylation of arachidonic acid into glycerophospholipids and its further remodeling into 2-AG (the "Flamand Pathway"). Unlike arachidonic acid, 2-AG is not metabolized by 5-lipoxygenase. Thus, 5-HETE-Glycerol and its oxidized metabolite (5-KETE-Glycerol) should not exist, unless their biosynthesis occurs via the Flamand Pathway. Given that 5-HETE is also acylated into glycerophospholipids, we postulated that 5-HETE-treated granulocytes would generate 5-HETE-Glycerol and possibly 5-KETE-Glycerol. **RESULTS.** 5-HETE-treated human neutrophils and eosinophils biosynthesized 5-HETE-Glycerol in a concentration- and time-dependent manner. Comparable data were obtained with 5-KETE-treated cells, which led to 5-KETE-Glycerol biosynthesis. Both monoacylglycerols were also detected when neutrophils were treated with arachidonic acid or A23187, indicating that endogenously formed 5-HETE/5-KETE also undergo the Flamand Pathway. We next assessed whether these new monoacylglycerols were found in vivo in joints from arthritic mice, a tissue containing high levels of 5-HETE. We did not detect 5-HETE-Glycerol but 5-KETE-Glycerol was found at comparable levels to 5-KETE. Initial in silico analyses indicated that these new lipids were ligands for cannabinoid receptors but this has not been clearly confirmed by wet lab data yet. **CONCLUSIONS.** Human granulocytes biosynthesize 5-HETE-Glycerol and 5-KETE-Glycerol. 5-HETE-Glycerol and 5-KETE-Glycerol thus represent two new arachidonic acid-derived metabolites derived from 5-lipoxygenase, with possible biological functions. We are now determining whether these metabolites are present in other inflammatory conditions that are enriched in 5-HETE/5-KETE as well as defining the cellular mechanisms by which they might modulate inflammation. Finally, the validation of the Flamand Pathway indicates that other fatty acid-derived metabolites could be synthesized by this new pathway, including those from the microbiota.

BLYS LEVELS ARE ASSOCIATED TO CLINICAL ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOUS PATIENTS

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B lymphocyte stimulator factor (BLyS) is produced by wide range of cells of the immune system, and has proven to be a key factor in the selection and survival of B cells. BLyS is an important factor in the pathology of Systemic Lupus Erythematosus; elevated serum levels of soluble BlyS are at increased risk of flare.

Objectives:

We aimed to analyze the association between the BLyS serological levels and clinical manifestations, as well as with disease activity in SLE patients.

Methods:

A cross-sectional, observational study in SLE patients (SLICC 2012 criteria) and healthy controls (HC) was performed. In SLE patients complete laboratory test, clinical evaluation and SLEDAI score was carried out. Serum concentration of BLyS was analyzed by colorimetric methods. Lupus patients were dichotomized as high and normaL BLyS levels based on BLyS levels above 2 SD of the mean in healthy controls.

Results:

166 SLE patients (86.7% female) participated in the study, with a mean age at diagnosis of 34 (14) years and a mean time of disease evolution of 16 (11) years, and 87 HC (67% female) with a mean age of 45 (14) years. The 32.5% of patients showed SLEDAI>6. The 57.8% were under glucocorticoid treatment, 47% under immunosupressants or biological therapy and 58.43% under antimalarials. BLyS levels were significantly higher in SLE patients (3133.8ng/mL) than in HC (2618.02ng/mL) (P=0.036), and 4600.72ng/mL was the cut-off point. The 12% of patients and the 3.4% of HC displayed increased BLyS levels. Higher BLys levels showed a significant association to elevated SLEDAI score (P=0.03) and were significantly associated to anti-dsDNA positivity (P=0,01), but showed no association with hypocomplementemia. The statistical analysis yield differences in belimumab treatment (P=0.002) and corticoid therapy (P=0.01) between patients with lower and higher BLyS levels. No influence of age at diagnosis, time of evolution and tobacco use in BLyS levels was observed.

Conclusion:

In our series we observed a 12% of patients with high levels of BLyS, and BLyS high levels showed a tendency to be associated to high SLEDAI score. BLyS levels are influenced by anti-dsDNA positivity, as well as to corticoid therapy and belimumab treatment.

Cadmium Induces Microcytosis and Anisocytosis without Anaemia in Hypertensive Rats

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Dietary cadmium (Cd^{2+}) intake is implicated in the pathogenesis of hypertension and anaemia, but there is a paucity of information regarding haematological changes in hypertensive conditions. This study, therefore, evaluated the effects of Cd^{2+} on blood pressure (BP) and haematological indices in Sprague-Dawley rat model. Three cohorts (n=10 each) of control and Cd^{2+}-fed male Sprague-Dawley rats were selected. Cd^{2+}-exposed rats received 2.5 or 5 mg/kg b.w. cadmium chloride via gavage thrice-weekly for eight weeks, while control animals received tap water. BP and blood flow were measured non-invasively from rat tails twice-weekly using a CODA machine, while weights were measured thrice-weekly. Haematological indices were assessed using the Cell-Dyn Emerald Haematology Analyzer. Data were reported as mean ± SEM, and statistically analyzed using One-Way Analysis of Variance. Bonferroni post hoc test was used for multiple comparisons. Cd^{2+}-exposure induced hypertension by significantly ($p<0.05$) elevating systolic, diastolic, and mean arterial BPs, pulse pressure, heart rate, and blood flow. Mean cell haemoglobin and mean cell volume were significantly ($p<0.05$) reduced, and red cell distribution width significantly ($p<0.01$) increased by exposure to 5 mg/kg b.w. Cd^{2+}. Mean cell haemoglobin concentration, haematocrit, haemoglobin, red blood cell, platelet, mean platelet volume, and white blood cell counts were unaffected by Cd^{2+}-exposure. Cd^{2+} induced hypertension with microcytosis, possible hypochromicity, and anisocytosis without anaemia, which may be precursor to microcytic anaemia and coronary artery disease. This study is important in Cd^{2+}-exposed environments and warrants further investigations into possible association between Cd^{2+} exposure and hypertension management and evaluating hypochromicity using blood smears.

Cathelicidin regulates goblet cell mucus secretion during *Citrobacter rodentium*-induced colitis

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Colonic goblet cells by secreting Muc2 mucin and specific proteins is critical for physically entrapping and expelling invading enteropathogens. Thus, is not surprising that *Muc2*$^{-/-}$ littermates exhibit increased susceptibility to attaching/effacing *C. rodentium* colonization. The colonic epithelium also secretes small cathelicidin peptide, which potentially interacts intimately with goblet cells and were presumed to accumulate within the sterile inner mucus layer as a simple antimicrobial peptide defense. In this study, we aimed to determine the effects of cathelicidin on mucin secretion in goblet cells during *C. rodentium*-induced colitis and the impact on the mucus barrier defense. For this, we used cathelicidin-deficient (*Camp*$^{-/-}$) mice, mouse colonoids and human colonic LS174T goblet cells to elucidate the mechanisms by which cathelicidin regulates goblet cell secretions. Our results showed *Camp*$^{-/-}$ littermates infected with *C. rodentium* displayed increased fecal shedding and epithelial colonization. *Camp*$^{-/-}$ littermates at the peak of *C. rodentium* infection (7 dpi) had a deficient mucin layer with fewer Alcian blue/PAS filled goblet cells and a reduction in fucose and N-acetylglucosamine (WGA^{+}) glycoproteins. By transmission electron microscopy (TEM), goblet cells in *Camp*$^{-/-}$ colons were swollen and retained a large number of mucus granules during *C. rodentium* infection. *C. rodentium* infected *Camp*$^{-/-}$ littermates showed impaired reactive oxygen species (ROS) production and a transcriptomic profiling associated with decreased ROS biosynthesis and an increase in ROS negative regulators. In mucin producing LS174T colonic epithelial cells, human cathelicidin LL-37 promptly induced the secretion of goblet cell-associated TFF3 and RELMβ, via a ROS-dependent mechanism. These findings revealed that mice lacking cathelicidin (*Camp*$^{-/-}$) were more susceptible to *C. rodentium* colonization caused by defective goblet cell mucus and mucin-associated protein secretion via a ROS-dependent mechanism. Importantly, cathelicidin regulated mucus secretion revealing a non microbicidal actions of this peptide with homeostatic properties on the colonic mucus barrier, critical in excluding luminal microbiota away from the epithelia to clear bacterial infections and restore gut homeostasis.

Characterising an Experimental Acute Lung Injury Model for ADAM17 Pathology

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Acute lung injury (ALI) is the most frequent distant organ dysfunction in acute pancreatitis (AP), with more than a quarter of patients admitted for AP eventually developing ALI. It has a high mortality rate and is the main cause of death in AP patients. We have previously shown that a disintegrin and metalloprotease 17 (ADAM17) plays a role in promoting AP using the well-documented cerulein-induced experimental AP model. This is via a form of ADAM17 mediated signaling known as interleukin-6 (IL-6) trans-signaling whereby ADAM17 cleaves membrane bound IL-6 receptor to produce its soluble receptor (sIL-6R). However, the role of ADAM17 in ALI is undetermined. To assess the role that ADAM17 plays in ALI, we have initially employed a single day experimental model, with mice receiving 7 intraperitoneal injections of cerulein (50ug/kg in 50ul PBS). We found that this model showcases early molecular changes of ALI in the lung, such as increases in mRNA expression of inflammatory mediators and protein expression of signaling pathways known to be involved in ALI and linked to ADAM17. In particular, IL-6 trans-signaling components, interleukin-6 (IL-6) and signal transducer and activator of transcription 3 (STAT3) was augmented in cerulein-treated wild-type mice. Moreover, cerulein treated *Adam17*$^{ex/ex}$ mice (>90% reduction in global ADAM17 expression) showed a reduction in proinflammatory mediators and signaling pathways implicated in ALI, suggesting a role for ADAM17 in ALI pathology. Taken together, the single day cerulein model is useful for preliminary studies in determining whether ADAM17 plays a role in ALI and in characterizing its early changes. Studies using a longer model is currently underway to further explore the changes seen.

Characterization of P2Y/X receptors and their dysfunction in the relaxation of pulmonary artery derived from patients with or without pulmonary hypertension of Group 3

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Pulmonary hypertension (PH) secondary to lung diseases (PH Group 3) is associated with the highest incidence and mortality. This pathology is frequently associated with an inflammatory response where nucleoside phosphates could be involved [1]. Adenosine triphosphate (ATP) and diphosphate (ADP) or uridine triphosphate (UTP) and diphosphate (UDP) are known to regulate vascular tone. However, the cells, the receptor subtypes, and the mechanisms underlying the responses to these nucleoside phosphates on human pulmonary arteries (HPAs) have not been fully characterized, either in preparations derived from non-PH or PH patients.

In this study, we evaluated the vasorelaxant or contractile responses of different nucleoside phosphates (ATP, ADP, UTP, UDP, ATPγS, Adenosine) in HPAs derived from PH Group 3 and Non-PH patients in the presence or absence of endothelium. A pharmacological study (organ bath system) was performed using selective purinergic receptor (P2Y/X) antagonists or agonists [2]. Moreover, we examined the contribution of nitric oxide (NO) and prostacyclin (PGI_2) pathways to ATP-induced relaxation of HPAs using inhibitors of these pathways (L-NOARG and indomethacin, respectively).

This study showed that ATP, UTP and ATPγS produced greater relaxations of HPAs than ADP , UDP or Adenosine . The relaxant responses were mostly blocked by either MRS2279 ($P2Y_1$), ARC118925 ($P2Y_2$), ATP (10 µM, $P2Y_4$) or AR-C118925 ($P2Y_2$) while MRS4062 (P2Y4 agonist) was inactive. In addition, on HPAs derived from Group 3 PH patients, ATP and ADP produced a less potent relaxation (~ 50 % less compared to control). UTP and UDP did not produce any relaxation, suggesting a uridine purinergic dysfunction on HPAs derived from Group 3 PH patients.

Together, our results indicate that the purinergic agonists inducing-relaxation are endothelium, PGI_2, and NO dependent. Moreover, we are currently able to identify two (2) purinergic receptors ($P2Y_1$, $P2Y_2$) involved in the relaxation, and we are currently investigating three (3) others ($P2Y_{11}$ $P2Y_6$, $P2Y_4$). Finally, our results show that these relaxations are significantly reduced in HPAs from Group 3 PH patients, particularly the UTP relaxation, probably due to $P2Y_2$ and $P2Y_4$ dysfunction and might be a possible pathological mechanism underlying Group 3 PH.

[1] Cai Z et al 2020, PMID : 32867554
[2]. Jacobson KA et al 2020, PMID : 32037507

CXCL4, a chemokine upregulated in systemic sclerosis patients, is pathogenic on TLR9-induced pDCs and B cells

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Systemic sclerosis (SSc) is a fibrosing disorder, characterized by a vasculopathy, an exacerbated inflammation and an autoimmunity, partly due to the aberrant production of IFNα by the plasmacytoid dendritic cells (pDCs) and to defective central B cell tolerance. Indeed, pDCs have been documented to produce large amounts of IFNα via the TLR7 and TLR9 signaling. Moreover, deficiency of *MYD88*, which mediates the function of both TLRs, results in a failure to silence autoreactive B cells. CXCL4 is one of the inflammatory molecules found increased in the serum of SSc patients and its level correlated with skin and pulmonary disease. Our objective was to investigate whether CXCL4 regulates TLR9 signaling in pDCs and B cells. pDCs and B cells were isolated from the blood of healthy donors (HDs) or SSc patients using BDCA4 and CD19-magnetic beads respectively. pDCs or B cells were then cultured with medium, TLR9 ligand (TLR9-L) alone or with CXCL4. Cytokines gene expression and secretion were analyzed. Delivery of TLR9-L was analyzed by Amnis and confocal microscopy. We demonstrated that pDCs from SSc patients spontaneously secreted CXCL4, which subsequently potentiated the production of IFNα from TLR9-induced pDCs. We demonstrated that the CXCL4 formed nanoparticles with DNA, which promoted its uptake and skewed its delivery from the late endosomes to the early endosomes. This is concordant with our previous data that show that the intracellular trafficking of the TLR9 ligand in human pDCs is important. Indeed, IFN was produced from pDCs when the TLR9-L was localized in the early endosomal compartment where IRF7 is engaged. Surprisingly, we observed that CXCL4 abrogated TLR9 response in human B cells. CXCL4 prevented the delivery of TLR9-L to the late endosomes, where the signaling occurs in B cells. CXCL4 *in vivo* expression led to defective TLR9 responses and increased the number of polyreactive B cells, hence impeding central B cell tolerance. Our data provide evidence for a novel mechanism by which CXCL4 superinduces the interferon production by TLR9-induced pDCs. CXCL4 also impairs central B cell tolerance by altering the intracellular trafficking of TLR9 ligands, hence inhibiting TLR9 response which is required for the removal of developing autoreactive B cells. Taken together, our data shows a pathogenic role of CXCL4 in both pDCs and B cells.

Development and Application of a LC-MS/MS Method for Monitoring CFTR Modulators in Biological Fluids: A Step Forward in Cystic Fibrosis Treatment

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Summary: We have developed a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) based method for monitoring the concentrations of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) modulators: elexacaftor (VX-445), tezacaftor (VX-661), and ivacaftor (VX-770) in biological fluids from individuals with cystic fibrosis. This innovative methodology facilitates swift and sensitive quantification of CFTR modulators from minimal samples, offering significant benefits in practical applications.

Keywords: Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), drug monitoring, clinical pathology

Introduction Cystic fibrosis (CF) is the most prevalent life-shortening genetic disease in the Caucasian population, affecting approximately 100,000 individuals globally. In CF, mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene lead to a malfunctioning CFTR protein. Therefore, CF patients produce a thick and sticky mucus in the lungs, gut, pancreas, and the reproductive tract, leading to progressive respiratory function loss, poor growth, and infertility. Over the past years, the landscape of CF has significantly changed with the discovery of CFTR modulator drugs targeting the basic CFTR defect. These include the triple combination of elexacaftor, tezacaftor, and ivacaftor (ETI), which is currently the standard care for approximately 90% of CF patients. Consequently, there is a growing demand for monitoring ETI concentrations in biological fluids to support precision-based therapies in practical scenarios, including monitoring ETI in pregnant CF women and brestfed children. In this report, we introduce a novel LC-MS/MS-based quantification process for ETI in biological fluids, designed for rapid, sensitive, and minimally invasive drug monitoring**.**

Methods Elexacaftor (VX-445), tezacaftor (VX-661), and ivacaftor (VX-770) were detected and quantified in 100 µL of plasma or breast milk collected 12 hours after the last daily intake of ETI. Following protein precipitation, 10 µL of samples were injected into an Agilent 1260 Infinity II HPLC system equipped with a C18 (50 x 2.1 mm, 18 µm, Phenomenex) column using a gradient of acidified water/acetonitrile mobile phase (from 55:45 to 65:35 in an 8-minute run). The Agilent Ultivo QQQ mass spectrometer was equipped with a Jet Stream electrospray ionization (ESI) source and operated in positive mode. Standard curves and quality controls (QC) were obtained by spiking a pool of blank matrix with an analytical standard and internal standard (all from Cayman Chemicals). The 8-point calibration curve, ranging from 0.39 to 50 pg/µL, included the lower limit of quantitation/detection, which were calculated according to the EMA's guidelines**.**

Results Our findings indicate that VX-445, 661, and 770 were present in plasma and breast milk at ng/mL concentrations, while 100-200 lower concentrations were measured in plasma from an infant 6 hours post milk feed, suggesting maternal-newborn transfer of the 3 components of ETI.

Conclusions These findings demonstrate that the methodology developed and employed here is a significant advancement over previously published methods as it enables quantification of ETI from a small volume of biological sample, thereby minimizing the impact of withdrawal procedures, and with a linear dynamic range of pg/mL. Thus, it can be useful for real-life, post-marketing monitoring of ETI in patients with CF, including pregnant women, mothers, and infants. Our results also suggest that CFTR modulators can pass and remain chemically stable in breast milk, from where they can be absorbed by infants. While this can be advantageous for treating infants with CF early after birth, there have been cases of serious side effects in newborn children exposed to ETI prematurely. Hence, these findings highlight the importance of having a rapid, sensitive, and reliable method for monitoring ETI in biological samples.

Development of an analytical method to quantify commendamide and its congeners by tandem mass spectrometry.

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INTRODUCTION. An increasing body of evidence indicates that gut microbiota has a significant impact on human health. Indeed, the gut microbiota is the source of several bioactive metabolites. Its taxonomic composition can be dysregulated (dysbiosis) in diseases, notably metabolic syndrome. Some bacteria from the genus *Bacteroides*, a genus that is increased in obesity, can biosynthesize commendamide, which is an acylated glycine (*N*-3-hydroxy-oxohexadecyl-glycine). Thus, commendamide shares structural similarities with *N*-oleoyl-glycine, an *N*-acyl-amine that is part of the endocannabinoidome. Commendamide could thus represent a potential pharmacological target in dysregulated states linked to the endocannabinoidome, such as obesity and inflammation. In order to fully understand the role(s) of commendamide, we first need to develop an analytical method allowing its quantification in numerous biological matrices, notably feces.

OBJECTIVE. To develop an analytical method to quantify commendamide and its congeners by tandem mass spectrometry.

METHOD. Commendamide and and commendamide-d_2 were syntesized as described previously by Villano et al. Oleoyl-commendamide, palmitoyl-commendamide and their deuterated counterparts were synthesized using a similar strategy. We optimized the detection of the componds and developed a liquid chromatography method allowing the analysis of the compounds. Three extraction methods were also compared allowed us to analyze biological matrices, notably mouse feces and samples from a Simulator of the Human Intestinal Microbial Ecosystem (SHIME®).

RESULTS. Commendamide and its congeners were better detected in the negative mode ($M-H^-$) rather than the positive mode ($M+H^+$). Compound extraction was best achieved using a liquid-liquid extraction with acidified methanol and chloroform. Reproducibility experiments will be presented. Finally, commendamide and some of its derivatives were found in both feces and SHIME® effluents.

CONCLUSIONS. Our analytical method allows the detection and quantification of commendamide and its congeners. We are now focusing at establishing if there is a link between commendamide levels and *Bacteroides* species in selected samples.

Development of an Assay to Identify Novel Inhibitors of Neutrophil Extracellular Trap Formation (NETosis)

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NETosis is a specialized mechanism of cell death, achieved through the formation of neutrophil extracellular traps (NETs). NETs are lattices of extracellular fibers formed from released nuclear chromatin and cellular granular peptides, which can immobilize and destroy invading micro-organisms. However, aberrant formation or accumulation of NETs causes inappropriate activation of the host immune response and can contribute to the pathogenesis of several diseases, including diabetes, rheumatoid arthritis, and COVID-19.

The development of inhibitors that directly target NETs (e.g. DNase 1) or inhibit upstream activation and signaling events provide an attractive therapeutic approach to alleviate immune-inflammatory disorders. Ongoing commercial activity in this field includes the Phase 1 trial of a first-in-class anti-histone therapeutic CIT-013 (Citryll), and Brensocatib (Insmed Inc.), a DDP-1 inhibitor, undergoing Phase 3 trials for non-cystic fibrosis bronchiectasis.

A high-throughput compound screening platform was developed at Sygnature to quantify NETosis and differentiate NET formation from other cell death pathways such as apoptosis or necrosis. This was achieved using human primary neutrophils sourced from the in-house blood donor panel, in conjunction with IncuCyte® ZOOM live cell imaging and ImageXpress confocal microscopy platforms. Multiplex cell imaging assays were established to analyze the nuclear and plasma membrane morphology combined with fluorescent tagging of nuclear and extracellular DNA fibers. This approach enabled the quantitative detection of NET formation and the determination of literature inhibitor activity, giving scope for new targeted drug discovery programs. Furthermore, neutrophil reactive oxygen species (ROS) production, an important mediator in the NETosis pathway, was detected using a cytochrome C reduction assay, and inhibited by the MAPK-p38 inhibitor SB203580. To enable higher throughput screening, the NETosis assay was successfully transferred to a differentiated HL-60 human leukocyte cell line, enabling more efficient screening to help identify novel NETosis inhibitors.

Dexamethasone and TAK-242 reduce inflammation in the LPS-induced acute kidney injury (AKI) mouse model

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Acute Kidney Injury (AKI) is a severe pathological condition characterized by rapid onset and high mortality rates. It is often associated with the release of inflammatory cytokines, instigating extensive tissue damage, particularly in vital organs such as the kidneys. Clinical investigations have revealed elevated protein levels of proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, in the bloodstream of AKI patients. Lipopolysaccharide (LPS) can induce robust inflammatory reactions, leading to the release of multiple inflammatory cytokines. In this study, we examined the effects of Dexamethasone and TAK-242 on LPS-induced AKI in male C57BL6 mice.

Our LPS mouse model involved a single intraperitoneal (IP) LPS administration (0.3 mg/kg), with samples collected at 4h post-LPS. IL-6, IL-1β, TNF-α, IFN-γ were assessed by MSD, while MCP-1, CRP-1, and NGAL were evaluated by ELISA. Gene expression of CCL5, CCL2, KIM-1, podocine, and IL-6, IL-1β, TNF-α, IFN-γ was evaluated by qPCR. Plasma creatinine and urea levels were measured by COBAS and drug levels were quantitated using LC-MS.

LPS administration resulted in increased kidney levels of IL-6, IL-1β, TNF-α, IFN-γ, MCP-1, CRP-1, and NGAL compared to the control group. Both Dexamethasone and TAK-242 significantly reduced these levels ($p<0.001$), with NGAL being unaffected by Dexamethasone. TAK-242 reduced LPS-induced upregulation in IL-6, IL-1β, CCL2, CCL5, and KIM-1 gene expression, while Dexamethasone reduced IFN-γ, IL-6, KIM-1, and IL-1β gene expression. Plasma creatinine and urea levels, elevated in LPS-induced animals, remained unchanged with treatment. Dexamethasone level was twice higher in the kidney than in the plasma, whilst TAK-242 was undetectable in both at termination.

In summary, LPS injection induces cytokine release, leading to a subsequent decline in kidney function. Understanding LPS-induced kidney responses may help to investigate potential therapeutic targets for conditions characterized by excessive inflammation. LPS-PK:PD models serve as valuable translational tools, guiding the development of therapies for inflammatory diseases and ultimately improving patient outcomes.

Dextran Sulphate Sodium (DSS)-induced Ulcerative Colitis Model in Mice: Comparing the Effects of Two Therapies on Clinical Signs and Colon Cytokines Secretion

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Inflammatory bowel disease (IBD), which affects millions of people world-wide, is a chronic inflammatory condition of the gut caused by microbiome dysbiosis as well as environmental and genetic factors. IBD can be broadly classified as ulcerative colitis (UC) and Crohn's disease (CD). Among the various animal models that are used to mimic human IBD, the dextran sulfate sodium (DSS)-induced colitis model is more widely used due to its simplicity and recapitulation of several features observed in human ulcerative colitis. In the present study, we compared the efficacy of two anti-inflammatory therapies in the DSS colitis model in mice by examining their effects on clinical signs and pro-inflammatory cytokines release in colon tissues. First, induction of colitis was evaluated using two different concentrations of DSS (2% and 3%). DSS was provided *ad libitum* in drinking water continuously from Day 1 to Day 10. Disease-related clinical signs such as weight loss score, stool consistency score and fecal blood score were measured daily, and a disease activity index (DAI) score was calculated by summation of the aforementioned three scores. A DSS concentration-dependent induction of colitis was observed in the mice as evidenced by relatively higher clinical scores with 3% DSS compared with 2% DSS. Based on relatively higher and optimal level of colitis induction with 3% DSS, we selected this concentration to examine effects of the two therapies. Effects of 5-amino salicylic acid and cyclosporine A, both of which are used clinically as well as in preclinical studies as positive controls, were evaluated. Both drugs were administered orally once daily from Day 1 to Day 10. While cyclosporine A (80 mg/kg) attenuated the colitis scores in the DSS-treated mice, 5-amino salicylic acid (75 mg/kg) was unable to exert any effects. Furthermore, at necropsy, colon tissues were collected from the mice to examine the effects of these two drugs on the pro-inflammatory cytokine levels. Similar to the in-life results, only cyclosporine A was able to block the release of pro-inflammatory cytokines in the colon tissues. In conclusion, these results suggest that cyclosporine A can be used as a positive control tool drug in the DSS colitis model in mice.

Dichotomous role of galectin-9 in modulating disease progression in murine models of colitis

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Inflammatory bowel disease (IBD) is a multifaceted disease involving the integrity of the epithelial barrier, the gut microbiome, and its mucosal immune cells. Therefore, investigating the regulatory mechanisms that command recruitment and infiltration of circulating leukocytes, without contributing to persistent inflammation, are gaining traction as potential therapeutic targets. Facilitating crucial interactions between the microbiota and the intestinal epithelium, glycans remain a facet of significant interest in the alteration of mucosal immunity. Specifically implicated in disease regulation, we investigated the impact of Galectin-9 (Gal-9) supplementation and deficiency, and the synergetic influence of Gal-3, on disease progression of murine Gal-3 knockout (KO), Gal-9 KO, and Gal-3/Gal-9 double KO models of colitis. The KO models with DSS-induced injury reduced the associated weight loss compared to the wildtype (WT) DSS injury model, suggesting a potentially pro-inflammatory role of both galectins. However, there was no notable negative impact of intraperitoneal introduction of Gal-9 and Gal-3 respectively into WT DSS injury models. Considering the extensive involvement of disrupted T-cell homeostasis in IBD, the impact of Gal-9 was examined on T-cell driven model of colitis. Curiously, the receipient Rag (-/-) mice which were supplemented with recombinant Gal-9 were found to have reduced intestinal inflammation and better a clinical outcome in comparison to the diseased control. This investigation unveils a double-edged functionality for Gal-9, perhaps hingeing on the initial injury trigger. Finally, our studies indicate no detectable differences in the cytokine profile of bone marrow-derived macrophages (BMDMs) from WT and Gal-9 KO mice, in response to pathogen-associated molecular patterns (PAMPs). However, human monocyte-derived macrophages supplemented with exogenous Gal-9 can markedly stimulate TNF-α release. When further stimulated with increasing concentrations of LPS, a dose-dependent increase in TNF-α was observed. While no such pattern of secretion of TNF-α was found with Gal-3 supplementation, modest reduction of IL-6 levels was noted. Therefore, our results indicate that while Gal-9 and Gal-3 may not be central drivers of colonic inflammation, they present as viable therapeutic targets for intestinal inflammation.

Effect of rebamipide on LPS-associated inflammation in patients with postcovid syndrome

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Low-grade "chronic" inflammation (LGI) during the process of reconditioning after COVID-19 undoubtedly plays an important role. One of the main problems of LGI, which poses a risk to human health, is a significant increase in the risk of cardiovascular complications in the postcovid period, which include death from cardiovascular disease (CVD). Aim: To evaluate the efficacy of the effect of the drug rebamipide on C-reactive protein (CRP) and major lipopolysaccharide binding systems (lipopolysaccharide-binding protein (LBP) and bactericidal/permeability-increasing protein (BPI) in postcovid patients with severe arthralgias. Methods. A prospective single-center study included 62 patients aged 46±5.6 years with a history of COVID-19, taking non-steroidal anti-inflammatory drugs (NSAIDs) for joint pain on the background of postcovid syndrome. The patients were divided into two groups. The first group (n=34) received rebamipide at a dose of 100 mg 3 times a day and omeprazole 20 mg daily for 28 days. Group 2 - 28 patients, received only omeprazole 20 mg daily for 28 days. All patients underwent clinical examination, anamnestic data collection and blood tests (ELISA) for levels of CRP, LBP and BPI. Results. Significantly higher levels of CRP and LBP were registered in the studied groups before the treatment compared to the control group ($p<0,05$). On the contrary, BPI index was significantly lower in the groups of patients with postcovid syndrome ($p<0,05$). In peripheral blood in group No. 1, which took rebamipide in addition to PPI, a significant decrease in CRP level (mg/L) was found to 3.75 [2.82; 4.21] and post-drug 2.05 [1.85; 2.62] ($p<0.05$), respectively ($p<0.05$). In the second group (omeprazole monotherapy), no significant changes were found before the drug 3.4 [2.56; 4.0] and after PPI course 3.52 [2.68; 3.9] ($p>0.05$). Conclusion. Rebamipide can potentially become a therapeutic tool for combating LGI driven by LPS in individuals who have undergone a new coronavirus infection and have signs of postcovid syndrome accompanied by an increase in peripheral blood CRP and LBP as part of LPS-driven LGI. This work was supported by the Russian Science Foundation under grant no. 23-15-20021, https://rscf.ru/project/23-15-20021/.

Effects of the Selective Estrogen Receptor Modulator Bazedoxifene on Human Neutrophils' Energy Metabolism and Functional Responses

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Selective Estrogen Receptor Modulators (SERMs) are pharmacological drugs that are prescribed to postmenopausal women to treat symptoms of estrogen deficiency and to prevent osteoporosis and breast cancer. However, these medications, such as bazedoxifene, have shown secondary effects like increased inflammation in the body, but little is known about their impact on immune cells. Neutrophils are critical initiators of inflammation that play specific roles modulated by favouring their energetic metabolism towards glycolysis. It has been shown that estrogen can modulate metabolic activity in neutrophils and that SERMs can differentially impact certain functions, like NETosis. Hence, we hypothesize that SERMs could induce inflammation by upregulating glycolysis in neutrophils and thus activating them. We collected neutrophils from healthy women and men and treated them with physiological doses of bazedoxifene. We assessed neutrophil viability by Annexin V and propidium iodide staining, inflammatory functions by measuring the production of chemokines, like CCL3/MIP-1α and CXCL8/IL-8, and their metabolic activity with MTS assays and an extracellular flux analyzer (Agilent/Seahorse Bioscience). We further performed treatments with and without LPS to mimic infectious conditions. Physiological doses of bazedoxifene do not compromise neutrophil viability after 24 hours of treatment. However, higher doses of bazedoxifene alone can increase chemokine production and release, while lower concentrations can prime neutrophils to release more proinflammatory chemokines when challenged with LPS. Interestingly, neutrophil sensitivity to the drug is sex-dependent, with male neutrophils reacting at lower doses than female neutrophils. Furthermore, the response to bazedoxifene occurs rapidly, as observed by an acute dose-dependent effect on glycolysis, with different dose responses seen between men and women. Overall, we have seen that bazedoxifene can modulate neutrophil energetic profiles and affect their anti-microbial activity. Understanding how SERMs interact with neutrophils and impact their inflammatory functions will help improve drug regimens that will consider the potential side effects that patients could encounter while using these drugs.

Expression of the CD41/CD61 "platelet" complex on neutrophils in heart failure patients

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Neutrophils are key players in the immune system and play a crucial role in inflammation. Neutrophils release pro-inflammatory cytokines and NETs (Neutrophil Extracellular Traps), which play an active role in the process of vascular thrombosis. The interaction of neutrophils with platelets is a major component of the thrombo-inflammatory phenomenon. This interaction may be more pronounced in certain pathologies with a pro-inflammatory profile, such as heart failure (HF). Recently, it was discovered that neutrophils in lung cancer patients can express the platelet protein complex CD41/CD61 (GPIIb/IIIa). The CD41/CD61 complex is known for its role in platelet adhesion and aggregation. The aim of our study was to demonstrate the expression of the CD41/CD61 complex on neutrophils and biological activity in healthy volunteers and HF patients. Neutrophils were isolated by density gradient and analyzed by flow cytometry and confocal microscopy to determine the localization and expression of the CD41/CD61 complex. Our preliminary data indicate the intracellular presence of this complex in 80%-90% of neutrophils, while it is only expressed between 8% and 13% on their extracellular membrane. Our results also demonstrate that the CD41/CD61 complex plays a role in neutrophil adhesion to the extracellular matrix, since its blockade with a CD41/CD61 monoclonal antibody decreases HV neutrophil adhesion induced by IL-8 (100 nM) by up to 76%. Meanwhile, the use of eptifibatide, an antagonist of the CD41/CD61 complex, at a concentration of 1.5 or 50 µg/mL reduced the IL-8-induced adhesion by up to 74% and 70% respectively. Our data suggest that the CD41/CD61 complex is not exclusive to platelets, and its expression on neutrophils may confer them pro-thrombotic properties. The inhibition of CD41/CD61 complex could lead to a treatment of neutrophil-regulated thrombosis in heart failure patients.

Extracellular ADP enhances the release of chemokines by fibroblasts

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Among many functions, the intestinal epithelium prevents the intrusion of microorganisms from the intestinal microbiota into the systemic circulation. However, in inflammatory bowel disease, increased permeability of intestinal epithelial cells (IECs) allows these microorganisms to entry into the lamina propria where they activate several cells via their microbe-associated molecular patterns (MAMPs) triggering proinflammatory responses. Activated, stressed, or injured cells release endogenous nucleotides such as ATP, ADP, UTP and UDP into the extracellular environment activating cell surface bound proinflammatory P2 receptors. In the lamina propria of the intestine, fibroblasts are among the first cells to respond to MAMPs as they reside beneath the IECs. In this work, we show a role for nucleotide signaling related to innate immune responses of fibroblasts. Fibroblasts isolated from healthy human colon and the mouse fibroblast cell line NIH/3T3 express innate immune toll-like receptors (TLRs) -3 and -4 that, when activated by polyinosinic-polycytidylic acid (Poly(I:C)) or lipopolysaccharide (LPS), respectively, trigger the release of the neutrophils chemoattractant CXCL1/KC (mouse) or CXCL8/IL-8 (human). Activation of TLR-3 and -4 in the presence of the general P2 receptor blockers suramine, RB2 and apyrase, reduces the release of CXCL1/KC and CXCL8/IL-8. We determined by qPCR that human primary colonic fibroblasts and NIH/3T3 express $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors that all respond to ADP. We showed by luciferin-luciferase that NIH/3T3 cells release ATP following the activation of TLR-4 and that ATPase activity is significantly higher than ADPase activity suggesting an accumulation of ADP at the cell surface upon TLRs activation. In agreement, we found that ADP enhances CXCL1/KC release by LPS-stimulated NIH/3T3 cells. In human primary colonic fibroblasts, blockade of $P2Y_{13}$ receptor with the specific antagonist MRS 2211 following the activation of TLR-3 with Poly(I:C) significantly reduced the release of CXCL8/IL-8 when compared to controls thus supporting the effect of ADP in NIH/3T3 cells. Taken together, our preliminary results suggest a role for ADP in promoting the innate immune responses of fibroblasts.

Fibrinogen is one of the major proteins which undergoes reactive nitrogen species-mediated damage in human plasma

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Inflammation is associated with reactive nitrogen species generation (e.g., peroxynitrite) and the subsequent nitration of the constituent tyrosine residues within proteins, generating 3-nitrotyrosine residues. To assess if endogenous nitrated proteins can be measured in human blood, it is important to select the correct blood processing method. Processing to provide serum is a popular choice in both research and routine biochemical analyses, where coagulation results in the removal of fibrinogen. In the present study, 3-nitrotyrosine-containing proteins were detected and identified in healthy human plasma and serum samples by both native and reducing SDS PAGE with western blotting. Western blots using an anti-nitrotyrosine antibody, following native PAGE, revealed that most of the nitrated protein was in a single band, present in plasma but barely detectable in serum. This protein band was identified as fibrinogen using an anti-fibrinogen antibody. Further investigations using reducing SDS PAGE followed by western blotting, revealed that commercially sourced fibrinogen, from human plasma, contained endogenous 3-nitrotyrosine residues. Under reducing conditions, the β subunit of fibrinogen (52 kDa) was seen to be the most susceptible to nitration of the three fibrinogen subunits, in both the commercially sourced fibrinogen and in freshly sourced plasma samples. Nitrated albumin represented only a minor constituent of the plasma nitroproteome. In conclusion, nitrated fibrinogen was observed as a major constituent of the nitroproteome in human blood plasma. We propose that analyses of non-erythrocytic blood protein nitration are optimally conducted using plasma.

Keywords:
Plasma, serum, 3-nitrotyrosine, fibrinogen, nitrated protein, nitroproteome.

Gasdermin D mediates extracellular nicotinamide phosphoribosyltransferase (eNAMPT) secretion and release via NLRP3 inflammasome activation

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Damage-associated molecular pattern proteins (DAMPs) are innate immunity sentinels that are actively or passively released from tissue cells. Intracellular nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in nicotinamide adenine dinucleotide (NAD) biosynthesis and extracellular NAMPT (eNAMPT) is a potent DAMP amplifying innate immunity-mediated signals via Toll-like receptor 4 (TLR4) activation. Plasma eNAMPT levels are directly linked to disease severity in multiple acute lung and systemic inflammatory disorders. We utilized human THP-1 monocytic cells to mechanistically examine canonical NLRP3 inflammasome involvement in secretion/release of two DAMPs, eNAMPT and high mobility group box 1 protein (HMGB1). THP-1 cells exposed to Nigericin (triggering K+ efflux) showed rapid LDH release consistent with pyroptosis and NLRP3 inflammasome-dependent release of both DAMPs. eNAMPT and HMGB1 release was blocked by the NLRP3 inhibitor MCC950 and was abolished in caspase-1 deficient cells. Gasdermin D (GSDMD) cleavage is critical to GSDMD pore formation and induction of pyroptosis. Nigericin-induced release of eNAMPT and HMGB1 was abolished in GSDMD KO THP-1 cells. Lipopolysaccharide (LPS)/TLR4 signaling promoted GSDMD-dependent eNAMPT and HMGB1 secretion (abolished in GSDMD KO cells) but in the absence of LDH leakage and pyroptotic cell death. The pore-forming N-terminal GSDMD cleavage fragment did not appear in LPS-treated cells indicating eNAMPT/HMGB1 secretion/release is independent of GSDMD pore formation. We conclude that the NLRP3 inflammasome regulates LPS/TLR4-induced eNAMPT and HMGB1 secretion/release through lytic and non-lytic GSDMD functionality independently of pyroptosis involving an undefined mechanism.

GPER Ligands G1 and G15 Induce GPER-Independent Necroptosis: Unraveling Off-Target Mechanisms and Therapeutic Implications

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G-protein coupled estrogen receptor (GPER) is recognized as a novel therapeutic target in various diseases including cancer, cerebrovascular, metabolic, neurodegenerative, and chronic inflammatory diseases like arthritis, and inflammatory bowel disease. The anticancer effect of G1, a GPER agonist, has been investigated alone or in combination with pembrolizumab in patients with metastatic uveal melanoma. However, there are conflicting reports that the anticancer effect of G1 appears to be due to cytoskeleton destruction independent of GPER. In this study, we investigated the cytotoxic mechanism of GPER agonist G1 and GPER antagonist G15 in cancer cells and macrophages. Our findings revealed that both G1 and G15 induced concentration-dependent necroptosis in cancer cells, such as HT-29 human colon cancer and PC-3 human prostate cancer, and macrophages (TPA-differentiated THP-1 cells). The necroptosis induction was comparable to that observed with TNFα treatment, as demonstrated by FACS analysis with annexin V antibody/propidium iodide staining and the phosphorylation of RIP1, RIP3, and MLKL. G1- and G15-induced necroptosis and activities of p-RIP1, p-RIP3, and p-MLKL remained unaffected by GPER knockdown. Rather, G1- and G15-induced RIP1 phosphorylation was inhibited by the RIP1 inhibitor necrostatin-1 in a concentration-dependent manner. Additionally, the necroptosis triggered by G1 and G15 was effectively inhibited by taurodeoxycholic acid, an ER stress blocker, and vitamin E. On the other hand, G1 demonstrated the ability to attenuate the disruptive effect of TNFα on the intestinal epithelial barrier formation, while G15 did not. Taken together, GPER ligands, G1 and G15, induce cytotoxicity irrespective of cell type, and this effect is not mediated through GPER action. The current findings suggest that it is important to consider cytotoxicity issues when using GPER ligands G1 and G15 for GPER-dependent therapeutic effects.

Reactive nitrogen species-mediated damage to hemoglobin in the brain frontal lobes of patients with Alzheimer's disease

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Brain inflammation occurs in Alzheimer's disease (AD), and inflammation is often associated with reactive nitrogen species (RNS) generation. 3-Nitrotyosine, a product of RNS generation, was assessed in frontal lobe brain homogenates of AD or vascular dementia (VaD) patients and non-dementia (ND) control volunteers.

3-Nitrotyrosine-containing proteins detected by western blotting revealed a dominant 15 kDa nitrated protein band in both dementia (AD/VaD) and ND frontal lobe brain tissue. The 15 kDa band was identified by mass spectrometry as hemoglobin. The same band stained positively when western blotted using an anti-hemoglobin antibody. Image analysis of the fluorescence staining intensities of western blots indicated that there was a significant increase in the median extent of hemoglobin nitration (relative to the housekeeping protein) in the frontal lobe brain tissue from both the AD (n=10) and VaD (n=10) groups compared to ND volunteers (n=11; Mann-Whitney U test: AD v ND, $P < 0.0005$; VaD v ND, $P < 0.05$). The median extent of nitration within the hemoglobin band was higher in advanced AD patients (Braak stages 5-6) compared with early-stage AD (Braak stage 0; $P < 0.005$). In parallel, markers of nitric oxide [nitrite (NO_2^-) and nitrate (NO_3^-)], were measured by ozone-based chemiluminescence. The median NO_2^- levels (nmol/mg protein) were significantly higher in AD samples than in ND controls ($P < 0.05$). There were no statistically significant differences between the three groups when comparing median NO_3^- concentrations. Western blot image analyses of lysates from peripheral blood erythrocytes suggested that the median extent of nitration within the hemoglobin band was increased in AD compared to ND (n=4 in each group; $P < 0.05$). Females showed statistically significant increases in brain hemoglobin nitration and NO_2^- levels between the AD and control groups, but males did not. However, the group sizes in these sub-analyses were small.

In conclusion, the extent of hemoglobin nitration was increased in AD and VaD brain frontal lobe tissue compared with ND. We propose that hemoglobin nitration may be involved in the pathogenesis of AD.

Human Coronary Vascular Tone Induced by Neurotransmitters: Modulation by Omega-3

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Coronary artery diseases are characterized by chronic inflammatory status and endothelial dysfunction. This involves an increased production of neurotransmitters such as serotonin (5-HT) and acetylcholine. These changes are associated with effects on the vascular function by increasing vasoconstriction. Specialized pro-resolving lipid mediators (SPM), derived from omega-3 polyunsaturated fatty acids: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) play an active role in the resolution of inflammation. Recent results from our group show that DHA and metabolites (Resolvin D1, D5 and Maresin 1) reduce contractions of human coronary arteries (HCA) induced by PGE_2 . On the other hand, RvD5 and Mar1 production by human vagus nerve has been measured, their impact on the cardiac neuronal system remains unexplored.

The objective of this study is to investigate the impact of the omega-3 on the release and effects of neurotransmitters like acetylcholine and 5-HT in HCA.

The HCA were isolated from human hearts (n=6) after transplantation at Bichat Hospital and placed in an organ bath system. They were stimulated with different voltages to release neurotransmitters, before and after 1 or 18 h of incubation with omega-3. In order to evaluate the effect of DHA/EPA on exogenous neurotransmitters, a dose response curves with 5-HT and acetylcholine was realized. Vascular tone variations were analyzed.

Our results show that HCA contract after electrical stimulation, with an increased effect at higher voltages. The contractions resulting from this stimulation are attributed to a direct effect on smooth muscle cells and also to the neurotransmitter release, as they are partially blocked by tetrodotoxin (10 μM). DHA (0.1 mM) demonstrates the ability to reduce the contractions induced by stimulations at 10 and 30 volts by 56% and 31%, respectively. Additionally, exogenous neurotransmitters, such as 5-HT and acetylcholine induce contractions in HCA. Acetylcholine induced vasocontractions were reduced by DHA, while the serotonin-induced contractions remain unaffected by DHA/EPA.

Our preliminary results indicate that omega-3 may have an effect at the neuronal level in HCA, suggesting potential innovative therapeutic strategies.

Human eosinophils and neutrophils biosynthesize new lipoxygenase metabolites from monoacyl-glycerols and *N*-acyl-ethanolamines.

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INTRODUCTION. The endocannabinoids 2-arachidonoyl-glycerol (2-AG) and *N*-arachidonoyl-ethanolamine (AEA) are lipid mediators regulating many physiological processes, notably inflammation. 2-AG and AEA are respectively part of the monoacyl-glycerol (MAG) and *N*-acyl-ethanolamine (NAE) families. Thus, MAGs and NAEs are considered as part of the endocannabinoidome. Endocannabinoid hydrolysis inhibitors are being investigated as potential treatment in numerous conditions. This strategy will not only increase the levels of 2-AG and/or AEA, but also those of other MAGs and/or NAEs. Increasing MAG and/or NAE levels will likely increase the levels of their metabolites. Herein we investigated whether MAGs and NAEs were substrates for the 15-lipoxygenase pathway, which is strongly involved in asthma and its severity. We thus assessed if human eosinophils and neutrophils biosynthesized the 15-lipoxygenase metabolites of MAGs and NAEs derived from linoleic acid (LA), eicosapentaenoic acid (EPA), docosapentaenoic acid n-3 (DPA) and docosahexaenoic acid (DHA).

METHODS. We chemoenzymatically synthesized some putative 15-lipoxygenase metabolites of MAGs and NAEs containing LA, EPA, DPA and DHA and optimized their detection by LC-MS/MS. Human eosinophils and neutrophils were isolated from the blood of healthy volunteers and incubated with MAGs and NAEs at different concentrations and times.

RESULTS. Eosinophils, which express the 15-lipoxygenase-1, metabolized all the MAGs and NAEs to the expected 15-lipoxygenase metabolites. Human neutrophils, which might express the 15-lipoxygenase-2, also metabolized most of the MAGs and NAEs, but to a much lower extent than eosinophils. Importantly, some of the new 15-lipoxygenase metabolites we disclose were found in tissues from humans and mice.

CONCLUSIONS. We successfully showed that human eosinophils and neutrophils transform MAGs and NAEs into novel 15-lipoxygenase metabolites. How these new metabolites modulate the inflammatory cascade is now being explored as they could participate in the effects of endocannabinoid hydrolysis inhibitors *in vivo*.

Hyperactive popliteal afferent lymphatic vessels require activation of NADPH oxidase (NOX) and Endothelin (ET) pathways in female mice of the arthritic TNF$^{\Delta ARE/+}$ model.

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We examined the role of NADPH oxidase 2 (NOX2) and Endothelin (ET) pathways in the contractile dysfunction of popliteal lymphatic vessels draining inflamed ankles of TNF$^{\Delta ARE/+}$mice. Popliteal lymphatic vessels (pLVs) were isolated from female TNF$^{\Delta ARE/+}$ and wild type (WT) mice and mounted on a pressure myograph. Systolic (SD) and diastolic diameter (DD), amplitude, ejection fraction (EF), fractional pump flow (FPF), and contraction frequency (CF) were then measured in response to a stepwise increase in transmural pressure or increase in concentration of acetylcholine (ACh). Effects of NOX2 inhibitor VAS2870 (10 μM), free radical scavenger tempol (10 μM), and ET receptor antagonist bosentan (10 μM) were evaluated. Protein expression of phosphorylated (p-) and total (t-) eNOS in the vessels were determined by western blot. Differences were assessed by multiple t-test and one-way ANOVA followed by Sidak's posthoc test. Significant increase in the pLV CF (min^{-1}, 11.7±2.5 vs 4.9±0.4, $P<0.05$, n:10), SD (μm, 94.1±3.6 vs 77.5±6.2, $P<0.05$, n:10), and FPF (μm/min^{-1}, 3.9±1.4 vs 2.3±0.2, $P<0.05$, n:10) was measured in TNF$^{\Delta ARE/+}$ mice compared to WT. In the presence of VAS2870, tempol, or bosentan, differences in CF (min^{-1}, 8.0±1.3 vs 5.8±2.1; 11.3±2.1 vs 9.6±1.1; 9.3±1.0 vs 9.4±1.1, respectively) and SD (μm, VAS: 80.2±7.1 vs 71.8±5.6; tempol: 77.7±5.3 vs 76.8±6.8) were abolished. Additionally, administration of a concentration of ACh inducing a maximal response in WT vessels was not sufficient to inhibit lymphatic pumping in TNF$^{\Delta ARE/+}$ mice (min^{-1}, 4.7±1.7 vs 0.5±0.5, $P<0.05$). Significant increase in the protein level of p-eNOS, but not t-eNOS was observed in the TNF$^{\Delta ARE/+}$ mice (%, 450.4±143.8 vs 99.9±29.3, $P<0.05$, n: 5) compared to WT. These results indicate a correlation between increased activity of lymphatic vessels and activation of the endothelin pathway, together with increased production of eNOS-NO. Consequently, this leads to an increase in free radicals in arthritic TNF$^{\Delta ARE/+}$ mice. It is likely that such changes impact the effective drainage of inflammatory content from the joints to the pLN, thus influencing the immune response.

Identification Of A Novel Immunometabolic Target and Agonist For PLXDC2 For Amelioration of DSS Colitis In Mice

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PLXDC2 is expressed on the cell surface of macrophage, dendritic and specific mesenchymal and epithelial cells whose activation shifts cellular metabolism and reduces oxidative stress to rebalance the immune response and decrease inflammation. PLXDC2 activation improves disease severity in rheumatoid arthritis models (Tubau-Juni et al. J Immunol 206(Supp)), while loss of PLXDC2 exacerbates the severity of disease in DSS colitis (Tubau-Juni et al. Sci Rep 10(1)). PX-04 is a novel, first-in-class, orally active PLXDC2 agonist. Here, we report on the consequences of activating PLXDC2 using PX-04 in an acute DSS colitis model. Eight-week old mice were given DSS in drinking water for 7 days to induce disruption of the epithelial layer. Mice began once daily dosing with 20mg/kg of PX-04 24 hours after DSS initiation. Mice were weighed and scored daily for symptoms of disease (e.g. weight loss, diarrhea, rectal bleeding, rectal inflammation, pain, & overall behavior). Colons were collected and digested, and the resultant colonic lamina propria immune cell suspensions were isolated by Percoll gradient centrifugation. Cells were labeled with mixtures of extracellular and intracellular antibodies in a sequential live staining. Data were acquired using a FACS Celesta flow cytometer with FACSDiva software. Oral PX-04 treatment decreased the cumulative disease activity of mice challenged with DSS (FIG 1A). Immunologically, PX-04 affected both the adaptive and innate immune responses. First, PX-04 greatly decreased Th1 (FIG. 1B) and Th17 cells in the colon, while providing a slight increase to regulatory CD4+ T cells. A lower proportion of Natural Killer (NK) cells produced IFNγ. Meanwhile, the proportion of TNF producing dendritic cells was decreased by PX-04 treatment (FIG. 1C). In summary, PLXDC2 is expressed in cells relevant to IBD pathogenesis. PX-04, which binds to and activates PLXDC2, effectively decreases levels of effector T cells and myeloid cells, as well as overall disease severity in acute DSS colitis, warranting further investigation.

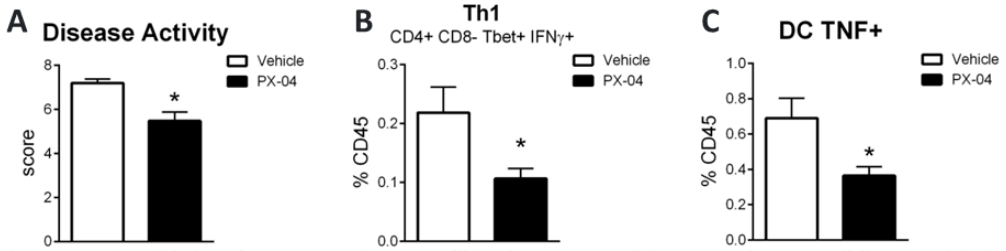

Figure 1: *In Vivo* Validation of PLXDC2 agonist PX-04 efficacy in acute DSS colitis. PX-04 reduces overall disease activity (A) as well as the proportion of Th1 T cells (B) and TNFα+ dendritic cells (DC)(C). *p<0.05.

Identifying novel effectors of interleukin-1β maturation in inflammatory macrophages by proximity-dependent biotinylation.

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INTRODUCTION: The autoimmune character of multiple sclerosis (MS) is well defined and the source of many treatments targeting immune cells of people living with the disease. Most immune-modifying drugs used for MS slow down the disease course, but generate an increased vulnerability to infections without curing MS. Using the experimental autoimmune encephalomyelitis (EAE) mouse model, we and others have demonstrated that interleukin-1β (IL-1β) is a key inflammatory mediator in the pathophysiology of EAE, as IL-1β-knockout mice are protected from the disease. To exert its proinflammatory role, IL-1β requires two different signals. A first one induces the transcription of the immature form of the cytokine, pro-IL-1β, and a second one activates the canonical inflammasome pathway, a large multiprotein complex responsible for the cleavage of pro-IL-1β into its bioactive form. We hypothesize that blocking all mechanisms of IL-1β maturation, whether inflammasome-dependent or not, will alter EAE progression and alleviate the symptoms. As a first step towards addressing this hypothesis, we aim to identify the proteases responsible for the maturation of IL-1β during inflammatory conditions using proximity-dependent biotinylation. **METHODS:** Proximity-dependent biotinylation takes advantage of a biotin ligase to attach a biotin molecule to every protein within proximity of a protein of interest. These proteins are then captured with streptavidin beads and identified using mass spectrometry. Here, we generated a plasmid coding for a doxycycline-inducible biotin ligase coupled to pro-IL-1β (TurboID-HA-pro-IL-1β) and expressed it in a RAW264.7 murine macrophage cell line using lentiviruses. RAW264.7 macrophages transduced with a plasmid coding for a doxycycline-inducible TurboID-HA-eGFP were used as a negative control. **RESULTS:** Immunoblotting analysis revealed a doxycycline-dependent expression of the construct in transduced RAW264.7 macrophages, and that the biotinylation capacity of TurboID increases over time, as anticipated. The expression and cellular localization of the construct were confirmed by immunofluorescence. Hence, we expect that this technique could help elucidate the IL-1β interactome through mass spectrometry. These findings could unveil novel therapeutic targets and pave the way for future research avenues aimed at discovering effective and safe treatments for MS patients.

IFN-β exposure and ARTS deficiency promote the generation of hyper-efferocytic Ly-6C$^+$ macrophages during the resolution of inflammation

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During the resolution of inflammation, Ly-6C$^+$F4/80$^-$ monocytes differentiate to Ly-6C$^-$F4/80$^+$ macrophages that exert efferocytic properties and consequently convert to IFN-β-producing macrophages. Here, we report that exposure to IFN-β, or TGF-β, or a deficiency in the pro-apoptotic protein ARTS, results in the conversion of mature macrophages to a rejuvenated Ly-6C$^+$F4/80$^+$CCR2$^+$ phenotype. This phenotype appeared exclusively in peritoneal resolution phase macrophages and not their unchallenged peritoneal, splenic or bone marrow counterparts. Moreover, IFN-β-triggered rejuvenated macrophages were hyper-efferocytic and expressed higher levels of the efferocytic receptor CD36. Inhibition of CD36 ligation resulted in complete abrogation of efferocytosis ex vivo in both mature and rejuvenated macrophages. Altogether, our findings indicate an unprecedented phenomenon in which IFN-β promotes macrophage rejuvenation and efferocytosis that are limited by ARTS-mediated apoptosis during the resolution of inflammation.

IGF-1 produced by microglia: a unique asset for facilitating central nervous system repair.

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Microglia are the resident immune cells of the central nervous system (CNS). Their involvement in phagocytosis of myelin debris and remyelination of the CNS is particularly relevant in demyelinating neurological pathologies such as multiple sclerosis (MS). In this context, microglia adopt phenotypes associated with damage, which are frequently linked to the expression of the *Igf1* gene. However, the mechanisms by which microglia aid in remyelination are still unclear. Given that Insulin-like Growth Factor 1 (IGF-1) is involved in cellular signaling, regulating proliferation, differentiation, migration, and survival, it is logical to question whether IGF-1 could play a pivotal role in CNS repair efforts by microglia.

Our hypothesis is that IGF-1 derived from microglia would be a mediator of microglial effectiveness in repairing the CNS. To test this hypothesis, we generated transgenic mice $Cx3cr1^{CreER}::Igf1^{fl/fl}$ in which microglia are conditionally invalidated in IGF-1 after tamoxifen treatment. We studied the effects of this mutation in combination with cuprizone treatment, during which the death of mature oligodendrocytes and subsequent demyelination are followed by robust microgliosis. After 5 weeks of cuprizone treatment followed by 1 week of recovery, *Igf1* gene deletion is associated with an increase in the amount of intact/non-phagocytosed myelin and a decrease in the number of oligodendrocytes in the corpus callosum. In parallel, the cKO mice show an increase in the number of proliferating microglia and those expressing the surface markers Cd11c and Ly9. Our results suggest that IGF-1 derived from microglia plays a role in the microglial polarization towards a reparative phenotype.

IgG and IgA autoantibodies against novel antigen specificities in systemic lupus erythematosus with differential underlying mechanisms heralding promise for early diagnosis

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Systemic lupus erythematosus (SLE) is characterised by excessive production autoantibodies (Abs), yet the antigen specificities remain elusive for most of these Abs. We performed a broad explorative screen of IgG and IgA antibodies against autoantigen specificities to gain insight into SLE pathogenesis and identify novel Abs that could enhance diagnostics. We analysed plasma samples from 289 patients with SLE and 196 age-/sex-matched healthy controls (HC) from the PRECISESADS project (NTC02890121). Samples were screened for IgG and IgA seroreactivity against a panel of >1,600 protein autoantigens using KREX-based i-Ome arrays. Differentially abundant Ab (DAAb) analysis revealed 14 IgG and 2 IgA DAAbs that were elevated in patients with SLE vs HC. Of those IgG DAAbs, anti-LIN28A (sen; spe; AUC: 0.77; 0.69; 0.80), anti-HBGB2 (0.65; 0.81; 0.79), anti-HMG20B (0.75; 0.71; 0.70), and anti-NRF1 (0.63; 0.80; 0.77) demonstrated the best ability to discriminate between SLE patients and HC. Of the IgA DAAbs, anti-LIN28A (0.65; 0.83; 0.80) and anti-SSB (0.64; 0.82; 0.78) demonstrated best metrics. Functional analysis of IgG DAAbs revealed 2 GO-term enrichments at the cellular component level: extracellular and cell surface, suggesting that autoantigens eliciting IgG are accessible at the cell surface or released by cell lysis. Functional analysis of IgA DAAbs revealed 17 GO-term enrichments related to DNA-binding transcription repressor activity, chromatin binding, transcription factor binding, and regulation of transcription by RNA polymerase II, suggesting that autoantigens eliciting IgA are enriched for nucleic acid binding. This study corroborated previously reported specificities (anti-HBGB2, anti-HMG20B) and revealed novel IgG and IgA (anti-LIN28A, anti-NRF1, anti-TGIF2, anti-SUB1, anti-PSIP1, anti-CCNB1) Abs described for the first time in SLE, with robust accuracy in distinguishing SLE from HC.

Immune complex recognition by FcγRIIa induces internalization, cytokine and extracellular vesicle release by Megakaryocytes

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Megakaryocytes, the parental cells of platelets, are diverse cells with immune cell characteristics and functional changes may affect the platelets they produce. They can express Toll-like receptors and the IgG-receptor FcγRIIa, which may enable the recognition of immune complexes by MKs, but this has never been shown. Here, we investigated if Megakaryocytes express functional FcγRIIa and if this facilitates the interaction with immune complexes and cellular responses.

MKs were obtained by 4-day differentiation of progenitor cells, isolated from bone marrow of healthy 7-15 weeks old C57BL6/J mice (FcγRIIa^{tgn} or FcγRIIa^{null}) and stimulated with microbial ligands and immune complexes (ICs). Extracellular Vesicles (EVs) and cytokines in supernatants and IC-uptake were analyzed by flow cytometry, ELISA and immunofluorescence microscopy.

MKs released various cytokines in response to TLR2 and TLR4 ligands. Only the cytokine MIP-2 was significantly increased in response to FcγRIIa stimulation with heat-aggregated IgG (ha-IgG). CD41+ and CD9+ MK-EV release was constitutive and refractory to TLR ligand stimulation. However, FcγRIIa^{tgn} MKs, but not FcγRIIa^{null} MKs, were found to bind and internalize ha-IgG, which induced cytokine and EV release. FcγRIIa^{tgn} MKs internalized mitochondrial ICs and coronavirus SARS-CoV-2 ICs, but only the latter induced MIP-2 and EV-release. These effects were not seen if MKs were incubated with SARS-CoV-2 in absence of antibodies or in presence of non-SARS-CoV-2 reactive IgG.

MKs may participate in adaptive immune responses through FcγRIIa expression. In particular, FcγRIIa activation can induce morphological changes, IC internalization and the release of EVs and cytokines by MKs. The nature of the response may depend on the type of IC as SARS-CoV-2 ICs modulated cytokine and EV-release by MKs in contrast to mitochondrial ICs.

Impact of the BPA-free copolyester Tritan on metabolism and functions of human neutrophils

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Some endocrine-disrupting chemicals (EDCs), such as the plasticizers bisphenol A (BPA) and bisphenol S (BPS), can affect not only endocrine functions but also the immune responses of various cells and tissues. Eastman Tritan™ copolyester, a novel plastic used as a replacement for BPA and BPS and manufactured utilizing three monomers, 1,4-cyclohexanedimethanol (CHDM), 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD) and dimethyl terephthalate (DMTP), can leach from products and could exert estrogenic activity. In this sense, various phthalates have been shown to increase inflammation and induce cellular metabolic changes. Since neutrophils are inflammatory cells that express hormone receptors and display a glycolysis-focused metabolism to fuel their antimicrobial functions, we hypothesized that Tritan™ compounds could modulate the metabolism and functions of neutrophils. We collected neutrophils purified from healthy women and men and treated them with Tritan™ compounds in the presence of pro-inflammatory cytokines to mimic inflammatory conditions. We assessed neutrophil viability by annexin V/propidium iodide staining, their metabolic activity with MTS assays and an extracellular flux analyzer, and their functions by evaluating reactive oxygen species production and cytokine secretion. We observed that Tritan™ compounds did not compromise neutrophil viability and glycolytic response. However, DMTP was found to reduce the respiratory burst induced by TNF and IL-1β, and all three Tritan™ monomers increased CXCL8/IL-8 secretion induced by these pro-inflammatory cytokines. Overall, these results suggest that Tritan™ compounds can modulate specific neutrophil functions. A better understanding of how Tritan™ compounds can impact the inflammatory functions of neutrophils will help determine whether Eastman Tritan™ copolyester may have potential adverse effects on our health or the health of future generations and if it is genuinely a safe replacement for other plasticizers such as BPA and BPS.

Inhibition of STAT3 Activation by JAK Inhibitor in the Synovial Tissues from the Hip Joint in the Early Stage of Rapidly Destructive Coxopathy

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Interleukin-6 signaling activates STAT3, leading to matrix metalloproteinase (MMP)-3 production. The hip joints with rapidly destructive coxopathy (RDC) show rapid chondrolysis, probably by MMP-3 increased in the synovial fluid in the affected hip joint. Currently, no information is available on STAT3 activation in the RDC synovial tissues. This study aimed to investigate STAT3 activation in the synovial tissues with joint destruction in the early stage of RDC. This study also investigated the effect of tofacitinib on STAT3 activation in the synovial tissues from the hip joint with RDC. Synovial tissues within 7 months from the disease onset were obtained from four RDC patients with femoral head destruction and high serum levels of MMP-3. The tissues were incubated with or without tofacitinib. Immunohistochemical examination was performed to detect STAT3 phosphorylation with anti-phospho-STAT3 antibody. RDC synovial tissues demonstrated the synovial lining hyperplasia with an increase of CD68-positive macrophages and CD3-positive T lymphocytes. STAT3 activation was found in the synovial tissues. The majority of phospho-STAT3-positive cells were the synovial lining cells and exhibited negative expression of macrophage or T cell marker. Treatment with tofacitinib resulted in a decrease in phospho-STAT3-positive cells, especially with high intensity, indicating effective suppression of STAT3 activation in RDC synovial tissues. Inhibitory effect of tofacitinib could work through the Janus Kinase/STAT3 axis in the synovial tissues in the early stage of RDC. Thus, STAT3 may be a potential therapeutic target for prevention of joint structural damage in RDC.

Intranasal administration of oxysterols promotes macrophage infiltration and reduces disease severity in SARS-CoV-2 infection

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Oxidised cholesterols, so called oxysterols, have emerged as novel regulators of inflammation in the lung during respiratory infections. We previously discovered that the oxysterols 25-hydroxycholesterol (25-OHC) and 7α,25-Dihydroxycholesterol (7α,25-OHC) are produced in the lung upon SARS-CoV-2 infection in mice and chemotactically attract infiltrating macrophages to the site of infection via the oxysterol-sensing receptor GPR183. However, the therapeutic implications of administering these oxysterols into the lung microenvironment during infection have not been investigated. Given that 25-OHC was reported to possess antiviral properties, we hypothesised that intranasal administration of these oxysterols may promote antiviral and immunomodulatory effects in the lung during SARS-CoV-2 infection.

Here we demonstrate that intranasal administration of 25-OHC but not 7α,25-OHC in C57/BL6 mice is antiviral and reduces the severity of SARS-CoV-2 infection-mediated disease. Furthermore, the increased concentrations of these oxysterols in the lung are associated with increased infiltration of macrophages. *In vitro*, we demonstrated that 25-OHC, but not 7α,25-OHC, exhibits antiviral activity in Calu-3 cells by blocking the early stages of the viral life cycle.

Together, our findings highlight the antiviral activity of 25-OHC in the lung during SARS-CoV-2 infection and demonstrate that enhancing local oxysterol concentrations can both promote macrophage infiltration and reduce inflammation into the lungs during SARS-CoV-2 infection.

Investigation of withaferin A as a novel therapeutic for neutrophilic asthma

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Asthma patients with airway neutrophilia tend to have more severe clinical disease compared to the eosinophilic asthma endotype. Despite the gap in effective therapeutics for neutrophilic asthma, much of the research on novel asthma therapies has focused on targets relevant to eosinophilic asthma. This highlights the compelling need for innovative anti-inflammatory strategies for neutrophilic asthma. Withaferin A (WFA), derived from the *Withania somnifera* plant, is a promising drug candidate for neutrophilic asthma. WFA has therapeutic effects in murine models of acute lung injury, pulmonary fibrosis, and other neutrophil-mediated diseases. Our published *in vitro* data demonstrate that WFA directly inhibits key inflammatory neutrophil functions and promotes timely apoptosis of primed neutrophils. The objectives of this study were to 1) evaluate the safety of pulmonary WFA delivery (via oropharyngeal aspiration, o.p.) in healthy mice and 2) investigate the effect of WFA o.p. on pulmonary neutrophil recruitment in an established murine model of neutrophilic asthma. Repeated administration of WFA via oropharyngeal aspiration (0.25-1 mg/kg on Days 0, 2, 4, 7, 9, 11, 14-16) was well-tolerated in healthy mice, with no evidence that local delivery of WFA into the lungs caused systemic disease or airway inflammation or cytotoxicity. Neutrophilic asthma was induced in separate cohorts of mice via OVA/LPS (50 µg/100 ng o.p.) sensitization on Days 0 and 7, followed by OVA challenge (50 µg o.p.) on Days 14-16. On Day 17, neutrophilic asthma mice treated with 1 mg/kg WFA o.p. had significantly less neutrophilic airway inflammation compared to vehicle-treated neutrophilic asthma mice. This blunting of airway neutrophilia by WFA occurred under both prophylactic (Days 0, 2, 4, 7, 9, 11, 14-16) and therapeutic (Days 14-16) WFA dosing schedules and was replicated across independent experiments. These data support the safety and efficacy of inhaled WFA as a novel therapy for neutrophilic asthma. Our ongoing investigations of WFA treatment for neutrophilic asthma includes refinement of inhaled delivery methods, optimization of dose, assessment of additional clinically relevant outcome measures, and evaluation in more chronic neutrophilic asthma models.

Itaconic acid modulates inflammatory response during sepsis, an intravital microscopy approach

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Metabolism fuels all biological processes and accordingly, alterations in immuno-metabolic pathways lead to, or accompany, numerous pathological conditions such as systemic inflammation. Continuous activation of the immune response, cell dysregulation and formation of neutrophil extracellular traps (NETs) lead eventually to robust inflammation, resulting in damage to the host cells and organs. We are therefore targeting immunometabolism as a potential anti-inflammatory therapy. In particular, we have been investigating itaconic acid, an intermediate produced as a consequence of metabolic shifts during macrophage activation. Itaconate was shown previously to impact macrophage functioning. Interestingly, recent reports indicate that itaconate can be also produced by a subset of highly mature neutrophils. In our *ex vivo* approach murine neutrophils were treated with 4-octyl itaconate (4-OI; itaconate derivative) or inhibitors of various metabolic pathways in the presence or absence of lipopolysaccharide (LPS). 4-OI dramatically inhibited NET formation *via* inhibition of hypoxia-inducible factor-1α (Hif-1α) and induction of nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase (HO-1) pathway. In the second approach, NETs were visualized in the liver sinusoids of living mice using intravital microscopy (IVM). IVM confirmed that 4-OI injected into endotoxemic mice reduces NETs without affecting neutrophil accumulation. Furthermore, we detected that itaconate reduced numbers of lymphocytes ($CD3^+$) in the liver vasculature. Moreover we detected impact of itaconic acid on Kupffer cells ($F4/80^+$; morphology), without affecting monocytes ($Ly6C^+$). Our results indicate that itaconic acid can metabolically modulates inflammatory leukocytes and their responses during systemic inflammation. The study was supported by National Science Centre of Poland, OPUS 22, 2021/43/B/NZ6/00782.

Juvenile Dermatomiositis under follow-up in the Adult Rheumatology Office

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Juvenile dermatomyositis (JDM) is a polygenic autoimmune interferonopathy with predominant involvement of the adaptive system. It is the most common inflammatory myopathy in childhood. The objective of our study is to review the clinical-analytical characteristics of patients referred to the transition consultation and assess their evolution.

14 patients were included (50% men) with a mean age (SD) of 6.53 (3.59) years at the onset of the disease and 6.92 (3.81) years at diagnosis. The median (Q1;Q3) follow-up of the patients is 18.68 (3.95;13.92) years

At diagnosis, 9(64%) patients presented positive ANAs, all had a speckled nuclear pattern. Regarding the specific antibodies, three AntiMi2, two AntiTIF1, two antiMDA5, one antiRO52 and one AntiSAE2.

Eight patients met definitive disease criteria according to Bohan and Peter. In the remaining six, the diagnosis was probable. All patients had CK levels above the laboratory's upper reference value at the time of diagnosis. Currently, five patients have elevated CKs levels.

Most patients have been treated with a combination of immunosuppressive drugs; Tacrolimus and methotrexate are the most used immunosuppressants with earlier initiation compared to other series published in the literature.

At the last visit, 85% of patients are in remission and 40% are untreated.

		Onset of the disease N=14	Transition visit N=14	Last visit N=14
CLINICAL MANIFESTATIONS		**N(%)**	**N(%)**	**N(%)**
Constitutional síndrome	General discomfort	6 (42.85)		
	Fatigue	8 (57.14)		
	Weightloss	2 (14.28)		
	Fever	1 (7.14)		
Muscular weakness		8 (57.14)	1 (7.14)	
Skin involvement	Gottron papules	8 (57.14)	3 (21.42)	1 (7.14)
	Heliotrope rash	6 (42.85)	1 (7.14)	1 (7.14)
	Nail involvement	1 (7.14)		
	Shawl sign	2 (14.28)		
	Malar Rash	3 (21.42)	1 (7.14)	1 (7.14)
	Calcinosis	3 (21.42)		
Gastrointestinal involvement	Dysphagia	4 (28.57)		
Pulmonar involvement	DLCO decline	1 (7.14)		
	Restrictive pattern	1 (7.14)		
Musculoskeletal involvement	Arthralgia	5 (35.71)	2 (14.28)	2 (14.28)
	Arthritis	2(14.28)		1 (7.14)

Linking NFE2L3 transcription factor activity to colon inflammation and colorectal cancer

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Early-onset colorectal cancer (EOCRC) is increasing at an alarming rate worldwide. Presenting in young individuals (18-49y) EOCRC is being detected at advanced stages of disease resulting in more unfavourable features as compared to late-onset CRC (>50y). Unlike late-onset CRC, EOCRC is highly prevalent in the distal/rectal parts of the colon. Sidedness, the location within the colon, has become an important prognostic and predictive factor in CRC patients. Also, oxidative stress has been associated with inflammatory bowel disease (IBD) and CRC. In earlier studies, we found that the loss of NFE2L3 in mice with colitis induced CRC reduced the numbers of distal/rectal tumors of the colon. Recently, our RNA-seq data from an acute inflammation mouse model showed that variance of genes regulated following was more prominent in the distal and mid colon and that the effect of knockdown of NFE2L3 was most defined in the distal colon as compared to the proximal colon. Our results revealed a role for NFE2l3 in oxidative stress signaling and in the inflammatory response in mice. We found that NFE2L3 stringently regulates a specific set of oxidative stress target genes including STAT1 and SLC7A11, which have been linked to IBD and CRC previously. We are currently validating the role of regulatory proteins in IBD and CRC by analysis of human tissue specimens obtained from IBD and CRC patients. Overall, our project aims to gain insights into the molecular mechanisms involved in colon inflammation and CRC by elucidating the role NFE2L3 is playing in colitis and colon carcinogenesis.

Lipid mediator profiling in human immune cells reveals potent 5-lipoxygenase inhibition by active components of *Panax Ginseng*

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The perennial plant *Panax Ginseng* has been used in traditional medicine in China and Korea for centuries. The preparations of this plant are known to have a wide variety of pharmacological effects, including anti-cancer, anti-diabetes, and anti-inflammatory activities. Although the anti-inflammatory features of the plant have long been described, little is known about the exact components that are responsible for this anti-inflammatory activity. The main active ingredients of *Panax Ginseng* are seemingly steroid glycosides present in the root of the plant, known as ginsenosides. Here, we present a systematic screening of 23 ginsenosides for their immune-modulating and anti-inflammatory properties by means of lipid-mediator profiling in classically activated macrophages.

Among a diversity of cyclooxygenase- and lipoxygenase (LO)-derived lipid mediators, several ginsenosides selectively inhibited the formation of 5-LO products, thus suppressing the formation of the chemotactic and pro-inflammatory leukotriene B_4 (LTB_4). Three clear structure-activity relationships are evident for ginsenosides to inhibit 5-LO product formation. Firstly, despite their highly similar structure, only PPD-type ginsenosides were able to inhibit 5-LO product formation. Secondly, the potency of PPD-type ginsenosides to inhibit 5-LO product formation was highly correlated with their lipophilicity ($R^2 = 0.92$). Lastly, based on the configuration of the carbon atom at position 20, two epimers of ginsenosides can be created. We observed only (20*S*)-epimers to potently inhibit 5-LOX product formation, whereas the (20*R*)-epimers were mostly inactive.

The complex and tight regulation of 5-LO activity in the cell allows for multiple mechanisms as point of attack for compounds that suppress 5-LO product formation. Direct inhibition of 5-LO itself by ginsenosides could be excluded, suggesting secondary mechanisms to be at play. Inhibition of cellular 5-LO product formation by ginsenosides was rapid (<1 minute) and irreversible. Interestingly, the aglycon PPD interfered with 5-LO subcellular redistribution necessary for enzyme activation and access towards substrate. Therefore, the aglycon PPD inhibits 5-LO product formation by means of interfering with 5-LO translocation, reflecting an effective strategy to interfere with LT biosynthesis. In conclusion, our study sheds light on (i) the bioactive anti-inflammatory ingredients of *Panax Ginseng,* and (ii) the underlying anti-inflammatory mechanisms targeting 5-LO product formation in innate immune cells.

Liver Circadian Rhythm: Impact on Mitochondria, Endocannabinoid System, and Immunology

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Environmental factors alter human physiology through two main factors: temperature (Tm) and photoperiod variance (Ppv). Tm directly increases the number of mitochondria along with endocannabinoid components in the metabolic systems such as liver, white, and brown adipose tissue. The endocannabinoid (eCB) system consists of enzymes and fatty acids with regulatory effects, namely on adipose and gut tissue/microbiome to brain and behavior. Exposure to constant light disrupts circadian rhythms, leading to alterations in gene expression and endocannabinoid molecule levels. In this study, we investigated the impact of disrupted circadian rhythms on gene expression and endocannabinoid levels in mice liver tissue. Our results revealed significant downregulation of Phospholipase C Beta 1 (PLCB1) and GPR110 in mice liver tissue following circadian disruption. To further elucidate the role of circadian rhythm in gene regulation, we generated a knockout cell line targeting the BMAL1 gene. Gene expression analysis in the knockout cell line showed consistent downregulation of PLCB1 and GPR110. PLCB1 plays a crucial role in calcium signaling by catalyzing the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). This enzymatic activity leads to the release of calcium ions from intracellular stores, such as the endoplasmic reticulum, into the cytoplasm, thus modulating various cellular processes, including gene expression, cell proliferation, and neurotransmitter release. Our results show an increased calcium level in mitochondria of Bmal1 KO cells. Also, Treatment with docosahexaenoylethanolamine (DHEA), an agonist of GPR110, resulted in decreased calcium levels in mitochonderia, suggesting a potential link between circadian rhythm, endocannabinoid signaling, and mitochondrial function. Overall, our findings suggest a complex interplay between circadian rhythm, endocannabinoid signaling, and mitochondrial function, with implications for lipid metabolism and immunological processes.

Long Chain Fatty Acids Reduce the Production of Inflammatory Markers in Bovine Endometrial Cells

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Endometrial cells play a key role in the control of the inflammatory response at postpartum, in addition to their physiological reproductive function. Endometrial cells express receptors for free fatty acids (FFA) such as FFA1 and FFA4 receptors. Both FFA1 and FFA4 receptors bind long chain fatty acids, such as linoleic acid (LA) and docosahexaenoic acid (DHA), respectively. In cows, linoleic acid increases in the blood during early postpartum period, however, its role on the inflammatory response in endometrial cells is unclear. On the contrary, DHA is added to the feed of postpartum cows, because DHA has been shown to have an anti-inflammatory effects on various cells. The aim of this study was to determine the inflammatory response-associated effects of LA and DHA in bovine endometrial cells.

Bovine endometrial (BEND) cells were cultured with linoleic acid (LA) or docosahexaenoic acid (DHA) for 30 min, then lipopolysaccharide (LPS) was added and incubated for 24 h. Interleukin-6 (IL-6), IL-8 and prostaglandin E2 (PGE2) were assessed by ELISA assay. The extracellular adenosine triphosphate (ATP) production was measured with a luminescent assay in supernatants of BEND cells incubated with LA for 15 s. Also, a possible effect of LA and DHA on changes in metabolic routes was assessed through metabolomic assay. The results showed that LA reduced the IL-6 and IL-8 production induced by LPS, but did not reduce PGE2. LA increased the extracellular ATP levels at 15 s of incubation. DHA reduced the IL-6 and PGE2 production induced by LPS, but did not affect IL-8 production. The metabolomic assay showed that LA and DHA induced changes in metabolites such as adenosine, arachidonic acid, stearic acid, and others.

In conclusion, both LA and DHA showed an anti-inflammatory effect on LPS-activated endometrial cells. Furthermore, LA and DHA induced metabolomic changes, which will be studied in detail to establish a link between immunity and metabolism. Understanding the interplay between immunity and metabolism will be useful to propose strategies based on fatty acids for adequate control of inflammatory processes in the endometrium. (Funded by Fondecyt No. 1200905)

Low-density neutrophils contribute to subclinical inflammation in patients with type 2 diabetes

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Type 2 diabetes (T2D) is characterized by low-grade inflammation. Low-density neutrophils (LDNs) represent normally less than 2% of total neutrophils but increase in multiple pathologies, releasing inflammatory cytokines and neutrophil extracellular traps (NETs). We assessed the count and role of high-density neutrophils (HDNs), LDNs, and NET-related activities in patients with T2D. HDNs and LDNs were purified by fluorescence-activated cell sorting (FACS) and counted by flow cytometry. Circulating inflammatory and NETs biomarkers were measured by ELISA (Enzyme Linked Immunosorbent Assay). NET formation was quantified by confocal microscopy. Neutrophil adhesion onto a human extracellular matrix (hECM) was assessed by optical microscopy. We recruited 22 healthy volunteers (HVs) and 18 patients with T2D. LDN counts in patients with diabetes were significantly higher (160%), along with circulating NETs biomarkers (citrullinated H3 histone (H3Cit), myeloperoxidase (MPO), and MPO-DNA (137%, 175%, and 69%, respectively) versus HV. Circulating interleukins (IL-6 and IL-8) and C-Reactive Protein (CRP) were significantly increased by 117%, 171%, and 79%, respectively, in patients compared to HVs. Isolated LDNs from patients expressed more H3Cit, MPO, and NETs, formed more NETs, and adhered more on hECM compared to LDNs from HVs. Patients with T2D pre-sent higher levels of circulating LDN- and NET-related biomarkers and associated pro-inflammatory activities.

Low-grade inflammation in postcovid patients may be caused by an imbalance of lipopolysaccharide-binding systems

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Currently, the pathophysiologic mechanisms of acute organ and system damage as a result of coronavirus infection have been sufficiently widely investigated; however, the mechanisms underlying the clinical manifestations of long-COVID have not yet been accurately described. The mechanisms of persistence of a number of symptoms in COVID-19 patients and the role of markers of systemic inflammation and endotoxinemia in it remains an understudied aspect and a promising area for further study. Aim of the study: to evaluate markers of systemic inflammation, endotoxin-realizing systems and intestinal permeability, as well as endothelial dysfunction in patients with post-COVID-19 at the sanatorium-resort stage of treatment. Methods. Thirty-two patients who had undergone coronavirus infection and were on sanatorium-resort treatment in the pulmonology department of the State Budgetary Institution of the Republic of Crimea "Academic Research Institute of Physical Methods of Treatment, Medical Climatology and Rehabilitation named after I.M. Sechenov" were examined. A control group (n=20) was also selected. All patients were analyzed peripheral blood for the levels of markers of systemic inflammation and endotoxin-binding systems: C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP) and bactericidal/permeability-increasing protein (BPI). Results. A statistically significant increase in the level of CRP (3.4 [2.56; 4.0] mg/L), LBP (18.46 [14.0; 25.5] ng/mL) and decreased BPI (1576 [276; 3588] pg/mL ($p<0.05$) was found in patients with postcovid syndrome, compared to the control group. Conclusion. Significant increase in markers of systemic inflammation and endotoxinemia in the group of patients with postcovid syndrome indicates an imbalance of endotoxin-binding and endotoxin-realizing systems in patients who have undergone coronavirus infection. It is necessary to further study the described markers to improve approaches to long-term personalized therapy of this category of patients. This work was supported by the Russian Science Foundation under grant no. 23-15-20021, https://rscf.ru/project/23-15-20021/.

Low-intensity inflammation in experimental metabolic syndrome and correction by polyphenolic grape products

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Polyphenols, such as those found in grape, and type 1 angiotensin II blockers, such as Azilsartan, can be used to help reduce the chronic inflammation and oxidative damage caused by metabolic syndrome (MetS). Polyphenols have antioxidant, hypoglycemic effects and nephroprotective properties. Azilsartan is a lipophilic molecule that can penetrate cells and stimulate the PPARy (peroxisome proliferator-activated receptor gamma) pathway, which improves carbohydrate and lipid metabolism.

The metabolic syndrome was modeling in 158 male Wistar rats, based on a fructose-feeding model. Group 1 received drug "Fenokor" with a total polyphenols' content of 181.53 g / dm3 at 45 mg/kg/day. Group 2 received grape seed concentrate with a total polyphenol content of 22.4 g/dm3 of 0.5 ml per day. Group 3 received Azilsartan at 1 mg/kg/day. The 4th group - MetS without correction. Different group 5 was the control without MetS.

Experimental modeling of MetS is associated with characteristic signs, such as abdominal obesity, hyperglycemia and dyslipidemia. As a result of the study, glucose level decreased by 50% in gr. 1 compared to gr. 4. Levels of TG, TC, and HDL-C recovered. In gr. 2, there was a significant decrease in TC and TG levels, although low HDL-C levels remained. In the gr.3, it was possible to achieve similar indicators to those of the control group. The activity of GLUT4 (glucose transporter type 4) and PPARγ was increased, while the activity of TLR4 (Toll-like receptor 4) and CRP (C-reactive protein) was decreased, the level of ceruloplasmin increased, and the peroxidase activity reduced in all groups with correction, compared with gr. 4. Morphometric analysis revealed a nephroprotective effect in animals against the background of correction with all drugs, especially Azilsartan.

Polyphenols' drugs have a diverse positive effect on blood glucose levels and lipids, as well as inflammatory and anti-inflammatory indicators. Our data suggest the potential of using these preparations to correct metabolic syndrome, including when used in conjunction with Azilsartan. However, more research is needed to confirm these findings.

Lysine 63-linked polyubiquitin free chains: a new second messenger involved in early GPCR signaling events

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G protein-coupled receptors (GPCRs), the largest family of receptors in humans, activate effector proteins to rapidly generate a multitude of second messengers such as cyclic adenosine monophosphate (cAMP), diacylglycerol (DAG), phosphatidic acid (PA), inositol 1,4,5-trisphosphate (IP3), calcium, nitric oxide (NO), cyclic guanosine monophosphate (cGMP) and reactive species of oxygen (ROS). Our research group has previously demonstrated the essential role of the ubiquitin ligase TRAF6 in the very rapid activation of the protein kinases TGFβ-Activated Kinase 1 (TAK1) and inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ), leading to an inflammatory response in vascular smooth muscle cells upon activation of the angiotensin II (Ang II) AT1 receptor. Following the covalent attachment of lysine 63-linked polyubiquitin (polyUbiK63) to substrates such as NEMO (IKKγ) or TAB2, TRAF6 controls the activation of IKKβ and TAK1 respectively. In vitro evidences also suggest that TRAF6 produces free unattached polyUbiK63 chains. Since TRAF6 is responsible for the very rapid activation of the inflammatory kinases TAK1 and IKKβ, we propose that following the activation of GPCRs belonging to the G_q/G_{11} and G_i families, there will be rapid and transient production of polyUbiK63 free chains, which will be found in immunocomplexes comprising the activated TAK1 and IKKβ kinases and leading to an inflammatory response. Using wild-type mouse embryonic fibroblasts and TRAF6-deficient cells, we show that the absence of TRAF6 reduces the anti-phospho signals of several effectors controlling the TAK1 and IKKb signaling cascades in response to lysophosphatidic acid (LPA) and thrombin (Thr). Furthermore, cells overexpressing domain that specifically binds free/unanchored polyubiquitin chains also show deficient signaling events in response to LPA stimulation. Our results demonstrate the pivotal role of TRAF6-mediated polyUbiK63 free chains in orchestrating the downstream inflammatory responses triggered by GPCR activation. These findings shed light on potential therapeutic targets for modulating the NF-κB inflammatory signaling pathways.

MCTR3 Improves Airway Epithelial Barrier Function and Decreases Pro-Inflammatory Responses Following Cigarette Smoke Exposure

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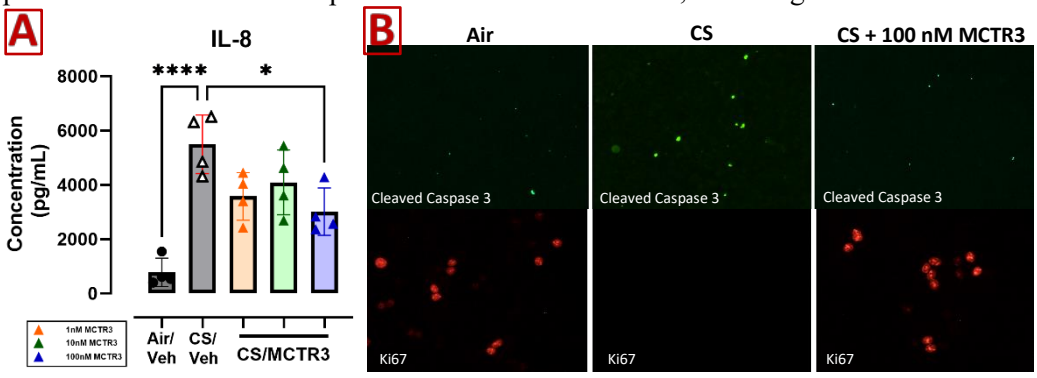
Rationale. Cigarette smoke (CS) is a potent pro-inflammatory stimulus that impairs normal mechanisms that resolve inflammation, leading to chronic inflammation that contributes to many diseases including lung cancer and Chronic Obstructive Pulmonary Disease (COPD). CS also directly damages the lung epithelium. Maresin conjugate of tissue regeneration 3 (MCTR3) is a novel specialized pro-resolving mediator (SPM) derived from dietary fatty acids that has the property of promoting tissue repair and regeneration in addition to promoting resolution of inflammation. Here, we investigated the ability of MCTR3 to promote repair of airway epithelium injured by CS.

Methods. Primary human small airway epithelial cells were differentiated at the air-liquid interface over 4 weeks and exposed CS for 30 min/day for 4 days. MCTR3 (1 to 100 nM) was added immediately after CS exposure on days 1-4. On day 5, culture media was collected, a FITC-dextran leak assay was performed to measure permeability of the differentiated cell layer, and the cells were fixed for immunofluorescence.

Results. CS induced production of the pro-inflammatory cytokine IL-8 and damaged the epithelial barrier, indicated by increased FITC-dextran in the bottom compartment. MCTR3 significantly reduced both IL-8 production and barrier leak (Fig. A and data not shown). CS induced apoptosis and decreased cell proliferation, as indicated by immunostaining for caspase-3 and Ki67, respectively. MCTR3 treatment after CS exposure restored Ki67 expression and blocked apoptosis (Fig. B).

Conclusions. MCTR3 protects the airway epithelium from CS-induced pro-inflammatory responses, cell death and epithelial barrier damage. MCTR3 demonstrates exciting, strong potential to be a novel therapeutic for CS-related diseases, including COPD.



Mechanisms and long-term consequences of neutrophil extracellular trap (NET) removal from vasculature

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During early stages of systemic inflammation neutrophil extracellular traps (NETs) released by neutrophils are engaged in pathogen trapping and immobilization. However, as the inflammatory response progresses, NETs persist in vasculature even if not needed any more. This is leading to bystander tissue damage and organ injury. Hardly anything is known about mechanisms and kinetics of NET removal in vivo therefore we aimed at investigating it. The process was studied during endotoxemia and NETs composed of neutrophil elastase (NE), histones and extracellular DNA (extDNA) were followed for 365 days in liver sinusoids with intravital microscopy (IVM). Protein NET components, unlike extDNA, were not detached from endothelium for months. Liver macrophages (Kupffer cells), but not monocytes, were mostly engaged in their removal but also neutrophils themselves participated in the process. Multiple receptors were engaged in this process, including TLR2 and TLR4 but also scavenger receptors. Although lipopolysaccharide (LPS) was used to induce inflammation, to avoid pathogen replication and survival in leukocytes, we detected self-renewal of NETs. The second wave of NETs was initiated by histones which triggered an inflammatory milieu, activated platelets and coagulation-related events, including factor VII-activating protease (FSAP) activity. Our study shows that complete removal of NETs *in vivo* is a very long process leading to a vicious cycle of NET formation. This finding explains detection of NET components in inflammatory disorders at their various stages and thus has a therapeutic potential. The study was funded by the National Science Centre of Poland (grant 2021/43/B/NZ6/00782).

Metabolic features of human neutrophils reprogrammed with inflammatory cytokines

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Neutrophils are no longer viewed as a homogenous population of short-lived cells. In this context, we identified a subset of inflammatory neutrophils from patients with autoimmune diseases that produce the peptidase inhibitor elafin. Such neutrophils are similar to a reprogrammed human population of long-lived (LL) neutrophils generated by a combination of cytokines associated with inflammatory diseases, *e.g.*, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF), and interleukin (IL)-4. These LL neutrophils exhibit characteristics of neutrophils and dendritic cells and produce elafin. Here, we characterized, using an extracellular flux analyzer, the metabolic changes in neutrophils exposed to IL-4, TNF, and GM-CSF, alone or in combination. Under inflammatory concentrations, TNF and GM-CSF, but not IL-4, increased the glycolytic response of neutrophils, while only TNF induced transient oxygen consumption (known as the respiratory burst). Reprogramming with TNF and IL-4 led to an increased glycolytic response without any effect on respiration. After 48h of reprogramming in culture, LL neutrophils exhibited an increased metabolic phenotype characterized by augmented glycolysis and respiration, both in resting state and following their activation with pro-inflammatory cytokines. To better understand the molecular mechanisms by which metabolic processes influence neutrophil functions, we went back to the transcriptomics of LL neutrophils (doi: 10.4049/jimmunol.2000852) and found an important modulation of mitochondria-associated genes. We detected higher transcript levels of the mitochondria-associated genes *SLC25A27*, *P2RX7*, and *PLPP3/PPA2B*, confirming LL neutrophils' distinctive metabolic features. These results suggest that LL neutrophils display an energized metabolic phenotype and could possess functional mitochondria capable of respiration to generate energy and sustain their functions. A better characterization of the metabolic features of these inflammatory neutrophils with pro-resolutive properties could represent new ways for therapeutic intervention in chronic inflammatory diseases.

Modulation of the inflammation/repair program of the intestinal epithelium by the dietary fiber rhamnogalacturonan

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Mucosal healing, the primary goal for IBD treatment, plays an important role in the re-establishment of the intestinal epithelial barrier function. Emerging evidence shows that key players of the inflammation initiation is also involved in the mucosal repair program. Our previous data showed that rhamnogalacturonan (RGal), a pectic polysaccharide, enhances the intestinal epithelial barrier function through TLR4 and PKC activation, and accelerates wound healing in Caco-2 cells and colonoids. RNAseq data and pathway analysis have indicated the involvement of the canonical nuclear factor κB (NF-κB) signaling pathway. We hypothesize that RGal drives an inflammatory gene expression profile to promote resolution of inflammatory phase and increase wound healing. Confluent Caco-2 and colonoids monolayers were apically treated with vehicle (media) or RGal (1000 μg/ml) for 6, 12 and 24 h. RNA was isolated and RNAseq was performed. Selected proteins were evaluated through electrochemiluminescence. For the neutrophil migration assay, Caco-2 monolayers were seeded on 24-well plates and treated with vehicle (media), TNF (10 ng/ml) or RGal (1000 μg/ml). Neutrophils (2×10^5) were added to 8 μm porous filter transwells and incubated for 4 h at 37 °C. Female and male mice with DSS-induced colitis were orally treated with RGal (10 mg/kg) for 7 days during the recovery phase. RGal treatment at different time-points induced changes in gene expression, upregulating the expression of inflammatory response genes responsible for neutrophil production and chemotaxis (CXCL1, CXCL2, CXCL3, CXCL5, CXCL8, G-CSF). RGal also increased CXCL8 family chemokines, confirming the gene expression data, and neutrophil migration by 98%. RGal treatment of male mice reduced the weight loss at later days, fecal lipocalin-2 levels and restored the colon length. In addition, RGal reduced the number of neutrophils when compared to DSS group but increased it compared to naïve group. These data suggest that RGal upregulates inflammatory genes and proteins downstream NF-κB activation. The RGal effect is mainly on neutrophil-dependent pathway since we observed increased neutrophil migration *in vitro* and *in vivo*. Our findings show that RGal can modulate the inflammation/repair program of the intestinal epithelium and this could lead to the resolution of intestinal inflammation and mucosal healing.

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Neutrophil extracellular traps from rheumatoid arthritis patients differentially activate myeloid cell sub-populations towards a pro-inflammatory profile, via mechanisms involving glycolysis and reactive oxygen species

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Activated neutrophils (PMN) expel neutrophil extracellular traps (NET), DNA/proteins fibers. Described as anti-microbial, NET can become immunogenic. Increased NET formation has been reported in rheumatoid arthritis (RA). We have previously shown that NET are pro-inflammatory on resting non-polarized M0 macrophages and this response was enhanced in RA patients. Here, we compared the pro-inflammatory activity of NET on cell sub-populations of the myeloid lineage, focusing on the effects of RA NET on target cells from healthy donors (HD), and we analyzed the involved mechanism. **Methods.** Blood PMN/PBMC were isolated from HD/RA patients. Monocytes were purified from PBMC. M0 macrophages were differentiated from monocytes or polarized to M1-like pro-inflammatory macrophages or M2c-like immuno-regulatory macrophages with cytokines. Pro-inflammatory dendritic cells (DC) were differentiated from monocytes with cytokines. NET were induced *in vitro* by PMA. Target cells were cultured with RA NET or LPS/R848, in the presence/absence of ATP to stimulate IL-1β secretion, or 2-DG/DPI to inhibit glycolysis/reactive oxygen species (ROS) production. Cell purity/phenotype/activation were estimated by flow cytometry. Cytokine secretion was measured by ELISA. **Results.** RA NET activated monocytes, PMN, macrophages and DC, leading to the secretion of pro-inflammatory cytokines. Particularly, NET triggered the secretion of RA-associated pro-inflammatory cytokines, but not of the immuno-modulatory cytokine IL-10. Importantly, even immuno-modulatory macrophages did respond to NET. In macrophages and PMN, NET induced the secretion of IL-1β, which occurred upon activation of the inflammasome in macrophages, whereas PMN did not require priming and produced IL-1β directly in response to NET. Finally, NET-mediated activation required ROS in monocytes, whereas it depended on glycolysis in PMN. **Conclusions.** Abnormal accumulation of NET in the extracellular space may be a major trigger capable to activate several myeloid cell sub-populations within a pathogenic pro-inflammatory response.

Neutrophil FcγRI: A New Player in Lupus?

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Systemic lupus erythematosus (lupus) is an autoimmune disease mainly affecting women of reproductive age. Its unpredictable manifestations can affect almost any part of the body. In lupus, neutrophils are held to drive overt inflammation through interactions with autoimmune complexes by producing reactive oxygen species and by releasing proteases that can cause tissue damage. Neutrophils recognize immune complexes via their Fc gamma receptors (FcγRs). In the present study, we observed that stimulation of whole blood from healthy volunteers with immune complexes specifically up-regulated FcγRI on leukocytes in circulation. This increase was particularly marked on neutrophils when compared to monocytes and lymphocytes. Immunofluorescence microscopy confirmed the specific up-regulation of FcγRI on neutrophils and further revealed a clustered distribution pattern for FcγRI. Exposure of isolated neutrophils to immune complexes resulted in the production of reactive oxygen species, which was prevented by blocking FcγRI. Because levels of autoimmune complexes can be higher in patients with lupus, we measured the surface expression of FcγRs on neutrophils in a cohort of patients, by flow cytometry. We observed that FcγRI was specifically elevated in lupus compared with healthy volunteers, although less pronounced in patients taking hydroxychloroquine, the first line of treatment for lupus. Preincubation of isolated neutrophils from healthy volunteers with hydroxychloroquine also diminished reactive oxygen species generation in response to immune complexes. This study identifies neutrophil FcγRI as a new and potentially relevant factor in the inflammatory component of lupus. Studies are in progress to delineate its precise functions for therapy purposes.

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Neutrophil-erythrocyte interactions *via* Siglec-E impact inflammatory activation and course of systemic inflammation

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Red blood cells (RBCs) are well known for their pivotal function in oxygen and carbon dioxide transport, however recently they are also recognoized for their major role in regulation of inflammation, including systemic inflammation. In sepsis, RBCs bind pathogens and store cytokines, but their plasticity is compromised and they release extracellularly hemoglobin/heme. Here we identify a modulatory effect of RBCs on murine neutrophils leading to their quiescence *via* the sialic acid-Siglec-E receptor pathway. We report that *ex vivo* murine RBCs increase neutrophil viability and release of IL-1beta but diminish lipopolysaccharide-induced Siglec E-dependent release of neutrophil extracellular traps (NETs). To further elucidate the impact of the interactions, we aimed at establishing a method of *in vivo* RBC imaging (anti-Ter-119 antibody) in the vasculature of live mice with intravital microscopy (IVM) at different stages of bacterial sepsis/endotoxemia. *In vivo* RBC-leukocyte interactions were clearly observed in the liver vasculature during sepsis (leading to either phagocytosis of RBCs or were transient in nature) and they exceeded those observed in healthy animals. Moreover, we applied phenylhydrazine-induced anemia model and blocked Siglec-E receptor in order to test whether these manipulations affect NET formation during sepsis. Prolonged neutrophil-RBC interactions, downregulation of NET formation and enhanced phagocytosis were observed as a result of RBC depletion. In the Siglec-E blocking approach, despite the lack of cell interactions, more NETs were formed both during systemic inflammation and in the accompanying hemolytic anemia. Our studies confirm the RBC-neutrophil crosstalk *via* sialic acids-Siglec-E axis but further studies are required to verify if these intrerations are beneficial or pathological for the sepsis outcome. The study was supported by National Science Centre of Poland, OPUS 22, 2021/43/B/NZ6/00782.

Novel IgG and IgA autoantibodies differentiating systemic lupus erythematosus from primary Sjögren's syndrome and systemic sclerosis

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Connective tissue diseases (CTDs) including systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS) and systemic sclerosis (SSc) frequently share clinical and serological features, rendering differentiation challenging. Currently used autoantibodies (Abs) either lack specificity (e.g., ANA) or sensitivity (e.g., anti-dsDNA). We performed a broad explorative screen of IgG and IgA Abs in SLE vs pSS/SSc to unravel disease-specific Abs. We analysed plasma samples from SLE (n=289), pSS (n=208), and SSc (n=187) patients from the PRECISESADS project (NTC02890121). Samples were screened for IgG and IgA seroreactivity against a panel of >1,600 protein autoantigens using KREX-based i-Ome arrays. Comparison between SLE and pSS revealed 10 IgG differentitally abadunt Abs (DAAbs; 8 elevated, 2 reduced) and 1 IgA DAAb (reduced). Of the elevated IgG DAAbs in SLE, anti-LIN28A (sen; spe; AUC: 0.71; 0.75; 0.78), anti-PCBP2 (0.56; 0.80; 0.74), anti-HMG20B (0.57; 0.81; 0.74), and anti-NRF1 (0.78; 0.64; 0.75) demonstrated best ability to distinguish SLE from pSS. Analysis of SLE vs SSc revealed 15 IgG (9 elevated, 6 reduced) and 4 elevated IgA DAAbs. Of the elevated IgG DAAbs in SLE vs SSc, anti-LIN28A (0.67; 0.84; 0.81), anti-HBGB2 (0.61; 0.85; 0.77), anti-HMG20B (0.66; 0.81; 0.79), and anti-NRF1 (0.64; 0.82; 0.77) demonstrated best metrics. Elevated IgA DAAbs in SLE vs SSc including anti-LIN28A (0.61; 0.91; 0.83), anti-HMG20B (0.78; 0.69; 0.80) and anti-NOL4 (0.68; 0.75; 0.76), showed good accuracy in differentiating between the two groups. This study corroborated traditional and previously described IgG Ab specificities (anti-LIN28A, anti-HBGB2, anti-HMG20B, anti-HNRNPA2B1) and identified novel IgG and IgA (anti-NRF1, anti-CCNB1, anti-LIN28A, anti-NOL4, anti-HMG20B) Abs described for the first time in SLE, with robust accuracy in distinguishing SLE from pSS or SSc.

Novel Resolvin D2 Epimer: A New Potent Bioactive Pro-resolving Mediator

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The production of specialized pro-resolving mediators (SPMs) during the resolution phase in the inflammatory milieu is key to orchestrating the complete resolution of the acute inflammatory response. Resolvin D2 (RvD2) exhibits potent pro-resolving functions in sepsis via activation of the GPR18 receptor (Nature, 2009, PMID: 19865173; JEM, 2015, PMID: 26195725). Here we uncovered a new resolvin in fresh human saliva. We identified it as epimeric to RvD2 after carrying out the first total organic synthesis of this new resolvin. The epi-RvD2 was produced by M2-like macrophages, M1-like macrophages and monocytes incubated with docosahexaenoic acid (5 μg) and zymosan (100 ng/ml) 45 minutes at 37°C. The physical properties, namely the chromatography and tandem mass spectrometry fragmentation pattern, of the biologically produced epi-RvD2 matched those of the newly synthesized material. We provide evidence that this new synthetic epi-RvD2 binds and activates the GPR18 receptor, in an equipotent manner as RvD2 (1-100 nM). In addition, the synthetic epi-RvD2 was shown to exhibit pro-resolving functions. Flow cytometric analyses revealed that topical application of epi-RvD2 (1 μg) 15 minutes prior to the addition of LTB_4 (1 μg) and PGE_2 (1 μg) significantly decreased the number of neutrophils in the mouse ear compared with the application of LTB_4 and PGE_2 alone. The synthetic epi-RvD2 increased M2 makers CD206 and CD163 on human monocyte-derived macrophages and enhanced efferocytosis of senescent red blood cells by M2-like macrophages. Also, increasing concentration of epi-RvD2 (0.1-10 nM) significantly increased phagocytosis of E. coli by human neutrophils in a time- and dose-dependent manner compared to vehicle alone. Together, these results establish this novel hydroxy epimer of resolvin D2 for its structure and potent pro-resolving functions.

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Nurr1 activation enhances ligand-dependent PPARγ1 activity in human macrophages

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Nurr1 is a member of the orphan nuclear receptor family NR4A which regulates different cellular processes such as differentiation, proliferation, survival, and inflammation. Nurr1 restricts the inflammatory response mainly through transrepression mechanisms, by interacting and inhibiting the function of transcription factors such as NF-κB p65. The peroxisome proliferator-activated receptor (PPAR)γ is another member of the nuclear receptor superfamily which is involved in the regulation of glucose and lipid metabolism, and shows anti-inflammatory properties. Nurr1 was found to be able to bind PPARγ, as well as to be recruited to the *PPARG* promoter in LPS-activated mouse microglial cells, although the functional relationship between Nurr1 and PPARγ remains unclear. In this study we aimed to investigate the association between Nurr1 and PPARγ1 in human macrophages. Pro- (GM-MDMs) and anti-inflammatory (M-MDMs) macrophages were generated by culture of human monocytes with GM-CSF or M-CSF, respectively. The protein levels of PPARγ1 and Nurr1 were elevated in GM-MDMs in comparison to M-MDMs, and their expression was positively correlated. This positive correlation was also detected in macrophages from adipose tissue samples. PPARγ1 activation in GM-MDMs with the agonist rosiglitazone did not modify Nurr1 expression. However, Nurr1 activation with the agonist C-DIM12 increased PPARγ1 protein levels through stabilization of PPARγ1 protein. Further, C-DIM12 decreased the number of PPARγ1 molecules phosphorylated at Ser84, which is a repressive mark for PPARγ transcriptional activity, similar to that observed with the rosiglitazone treatment. Co-exposure of GM-MDMs to C-DIM12 and rosiglitazone synergistically enhanced the transcriptional activity of PPARγ and the expression of two PPARγ target genes, *CD36* and *PLIN2*. Both PPARγ and Nurr1 agonists exhibited anti-inflammatory effects on GM-MDMs when administered alone, and they demonstrated some synergistic activity in combination. Collectively, these findings indicate that Nurr1 enhances PPARγ1 transcriptional activity, potentially through stabilizing PPARγ1 protein levels and by decreasing its repressive phosphorylation at Ser84. This novel mechanism highlights the role of Nurr1 targeting not only inflammation but also metabolic pathways in macrophages.

Omega-9 and 3 dietary intervention protects against inflammation in cigarette smoke-induced experimental COPD

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The impact of lipid mediators and the role of dietary polyunsaturated fatty acids (PUFAs) in chronic obstructive pulmonary disease (COPD), is poorly understood. Dietary modifications and interventions have been identified as a potential therapeutic intervention in COPD. We hypothesised that lipid mediator production is dysregulated in COPD and a diet high in monounsaturated fatty acid MUFA omega 9 (oleic acid) or omega-3 PUFA (alpha-linoleic acid) ALA would alleviate inflammation and lung function changes. Female, C57BL/6 mice (n=10) were fed a normal control diet (AIN93G), or high-fat diets (40% fat by energy) composed of predominantly monounsaturated (oleic acid) or omega-3 polyunsaturated (alpha-linolenic acid). Mice were exposed to nose-only cigarette smoke (CS) for (12 weeks) to induce experimental COPD. The hallmark features of COPD, including airway inflammation, histopathology (alveolar destruction), lung function (forced oscillations using Flexivent), and cachexia were assessed. Lipid mediators in bronchoalveolar lavage fluid and plasma were profiled using ultra-high-performance lipid chromatography-tandem mass spectrometry. In mice, MUFA and ALA diets reduced ($p<0.05$) CS-induced lung inflammation and protect against collagen fibrosis changes. MUFA and ALA diets didn't alter lung function in cigarette smoke-exposed induced experimental COPD. Ceramide and sphingomyelin were reduced in the MUFA diet compared to the control diet. Both the amount and composition of dietary fats impact disease features in COPD. Dietary increases in oleic acid and alpha-linoleic acid may be beneficial in improving health status in COPD.

$P2Y_2$ increases intestinal epithelial permeability that correlates with junctional adhesion molecule downregulation

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OBJECTIVE: We and others have found that intestinal epithelial cells express Nucleotide (P2) receptors that modulate inflammation. In inflammatory conditions, cellular damage occurs due to bacterial invasion, leading to the release of nucleotides into the extracellular environment. ATP and UTP, released by stressed intestinal epithelial cells (IECs), microbiota and other cells, activate $P2Y_2$ receptors, triggering the release of PGE2 by IECs. In this study, we evaluated the role of the $P2Y_2$ receptor in the DSS colitis model and its effect also on maintaining IEC homeostasis by regulating its integrity.
METHODS: $P2Y_2$-deficient (P2ry2-/-) and WT mice were treated with 3% DSS for 7 days. The disease score was assessed daily. On day 7, the colon was harvested for assessment of inflammatory markers. In addition, IECs were isolated from mouse colons and cultured. IECs were stimulated by TNF plus a TLR4 receptor ligand in the presence or absence of P2 receptor antagonists. The permeability and expression of tight junction proteins were evaluated by FITC-dextran and qPCR, respectively.

RESULTS: Mice deficient in the $P2Y_2$ receptor showed an increased disease activity index (DAI), indicating increased severity of inflammation in the DSS colitis model. Intriguingly, despite this proinflammatory phenotype, both in vitro and in vivo experiments demonstrated a decrease in the permeability of IECs in the absence of $P2Y_2$ signaling. Accordingly, analysis of stimulated IECs in the presence of a $P2Y_2$ antagonist revealed a significant increase in the RNA expression level of junctional adhesion molecule (JAM), suggesting a potential role of $P2Y_2$ in regulating epithelial barrier integrity.

CONCLUSION: Although the global absence of $P2Y_2$ in mice exacerbated intestinal inflammation in the DSS model of colitis, our data also point out the involvement of $P2Y_2$ in intestinal integrity. Preliminary analysis with IEC cultures provides further insights highlighting the role of $P2Y_2$ in reducing epithelial barrier integrity by affecting adhesion molecules. As $P2Y_2$ is expressed on both leukocytes and IECs further experiments will be needed to discriminate these effects. Therefore, our data suggest the involvement of $P2Y_2$ signaling in intestinal inflammation which warrants future investigations.

$P2Y_2$ nucleotide receptor and TLRs in human endothelial cells: A dynamic duo for IL-8 secretion

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The production and release of interleukin-8 (IL-8/CXCL8) by endothelial cells play a critical role in recruiting immune cells to the site of infection. In agreement, various stimuli such as pathogen-associated molecular patterns (PAMPs) and ligands for Toll-like receptors (TLRs) induce the release of IL-8, along with the release of various danger molecules such as nucleotides. Several studies, including the work of our research team, suggest the involvement of endogenously released nucleotides in inflammatory processes through the activation of their P2 receptors. In this research work, we investigated the role of extracellular nucleotides in PAMP-induced IL-8 release by human umbilical vein endothelial cells (HUVECs) expressing $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors. ELISA analysis of supernatants collected after stimulating HUVECs with TLR-specific PAMPs for 18 hours revealed significant IL-8 production in response to TLR3 (poly(I:C)) and TLR4 (LPS) ligands but not to TLR1/2 (Pam3CSK4) and TLR5 (flagellin) ligands. This response was inhibited in the presence of apyrase, an enzyme that hydrolyzes extracellular nucleotides, non-selective inhibitors of nucleotide receptors (suramin and RB2) as well as the specific antagonist of the $P2Y_2$ receptor (AR-C118925XX). Partial knock-down of the $P2Y_2$ receptor using specific small hairpin RNAs also resulted in a significant reduction in IL-8 release by HUVECs in response to poly(I:C) and LPS. Our results suggest that there is a release of nucleotides following the activation of TLR3 and TLR4. These nucleotides, in turn, activate the $P2Y_2$ receptor on the cell surface, leading to substantial IL-8 secretion. This highlights the critical role of the $P2Y_2$ receptor signaling in the modulation of poly(I:C)- and LPS-induced IL-8 secretion in HUVECs. Blocking $P2Y_2$ receptor could potentially prevent excessive inflammation and endothelial dysfunction.

Pilot study on the phenotype of tear leukocytes in seasonal ocular allergy and their potential impacts on ocular surface inflammation

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Upon prolonged eye closure at night, over hundreds of thousands of leukocytes can be collected from the ocular surface upon awakening, with polymorphonuclear neutrophils (PMNs) representing the main population. Under normal physiological conditions, these neutrophils, also known as tear PMNs, exhibit a distinct phenotype from circulating neutrophils, possibly due to their prior activation in the closed-eye environment. While leukocytes have been previously observed in tears of individuals suffering from ocular allergies, there is limited knowledge on tear PMNs and their potential role in ocular allergy. This study aimed to characterize the phenotype of tear PMNs collected from participants suffering from ocular allergy. Ten individuals experiencing symptoms of ocular allergy and ten without ocular allergy were recruited. Participants were asked to collect cells using a gentle eyewash on two consecutive days, after a full night of sleep in the morning on Day 1 (closed-eye tear PMNs) and at the end of the day on Day 2 (open-eye tear PMNs). After cell count, tear neutrophils were either activated with fMLP or left unstimulated, followed by staining with antibodies against degranulation markers (CD66b and CD63), activation markers (CD11b, CD54, CD62L), eosinophil marker (CD193), and aging marker (CD184), as well as with the fluorescent probe DCFH-DA to assess reactive oxygen species (ROS). Samples were analyzed by flow cytometry. Significantly more closed-eye tear leukocytes were collected from ocular allergy participants compared to healthy participants (p=0.015), while there was no difference in the number of open-eye tear leuckocytes. Furthermore, closed-eye tear PMNs from ocular allergy participants exhibited a less activated phenotype but a higher activation potential in response to fMLP, a response which correlated with their younger maturation state. There was no significant difference in the production of ROS, suggesting that oxidative stress may not be a key contributor to ocular discomfort associated with ocular allergy. Further research is needed to characterize the contribution of tear PMNs to the development or progression of symptoms of ocular allergy. Gaining a deeper understanding of the role of tear leukocytes in ocular inflammation may provide insights into the development of new therapeutical strategies.

Potent Novel Small Molecule Inhibitors of the Plasma Kallikrein Protease, a Key Regulator of the Pro-inflammatory Peptide Bradykinin

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The protease plasma kallikrein (PKal) cleaves high molecular weight kininogen which leads to the creation of the pro-inflammatory autocrine bradykinin. Bradykinin has long been implicated in a number of inflammation and edema associated disorders such as diabetic macular edema, hereditary angioedema, vasculopathy, neuropathy and cancer. Bradykinin, signaling through the B1 and B2 receptors, induces vasodilation, edema and sensitization of afferent nerves involved in pain.

Rezolute has created a novel suite of orally available small molecule plasma kallikrein inhibitors (PKIs). Our *in vitro* and *in vivo* preclinical evaluation funnel has been used to characterize the feasibility of these novel compounds to treat inflammatory disorders. These compounds exhibit IC50 values in the picomolar to single digit nanomolar range, and preliminary in vitro profiling of the lead candidates suggests an excellent pharmacology and drugability profile. One of the Rezolute compounds, RZLT – B, exhibits promising properties and is largely representative of the PKIs in development at Rezolute. RZLT – B has shown excellent plasma kallikrein inhibition both in purified PKal and human plasma PKal potency assays. Additionally, preliminary *in vitro* toxicity screens have highlighted a defined and understood safety profile. Pharmacokinetic studies have elucidated the consistent plasma exposure after oral dosing and *ex vivo* plasma potency studies have been used to characterize the *in vivo* inhibition of plasma kallikrein as a function of oral dosing. To better understand the use of RZLT – B in treating inflammation, a rat hind paw inflammation model has been used, with results comparable to the non-selective COX inhibitor indomethacin.

Though an established target for HAE, Plasma Kallikrein has only recently become a therapeutic target for inflammatory and leakage conditions due to the advent of small molecule inhibitors. The suite of novel plasma kallikrein inhibitors at Rezolute were created using a combination of rational design and computational chemistry approaches with a focus on a derisked safety profile and once daily oral dosing. Future studies with these compounds will seek to determine their application in Bradykinin mediated disorders.

Potential role of the hydroxyl carboxylic acid receptor type 2 (HCAR2) in microglia pathophysiology and pain implications

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The receptors of hydroxyl carboxylic acid (HCARs) belong to a group of GPCRs found on adipocytes, contributing to lipogenesis and fatty acid synthesis. The endogenous keton bodies butyrate and β-hydroxybutyrate (BHB) have been identified as ligands of HCAR type 2. Recent findings have shown the presence of HCAR2 on peripheral immunune cells and brain microglia, playing a role in modulating inflammatory responses and exerting a neuroprotective effect. Neuroinflammation occurs in many CNS diseases, including neuropathic pain. Indeed, resident microglia and infiltrating peripheral immune cells contribute to neurouflammation by releasing pronociceptive factors, cytokines and chemokines that further challenge the neuronal activity. Experimental model of neurophatic pain like chronic constriction injury (CCI) and spared nerve injury (SNI) showed an increased density of microglia cells in the ispilateral dorsal and ventral horn of the spinal cord and an overexpression of HCAR2 in spinal microglia and dorsal root ganglia (DRG) neurons. In these models the administration of BHB reduced tactile allodynia in neurophatic wild type mice but not in HCAR2 knockout (KO) mice. Moreover, the implementation of mid-term fasting regimen, capable of physiologically enanching endogenous ketone body production, ameliorated BHB alleviating mechanical allodynia in wild type mice but not in HCAR2 KO mice. In addiction, we found that in primary microglia colture, LPS challenge caused a morphological activation, incresed HCAR2 expression and intracellular level of COX-2 and IL-1β suggesting a proinflammatory phenotype. The pretreatment with MK1903, a potent full agonist of HCAR2, reduced the LPS-mediated microglia proinflammatory activation with a restoration of a resting phenotype. Due to these evidence, futher investigations are currently ongoing into potential role of HCAR2 in modulating microglia pathophysiology and widespread pain conditions induced by early life stress as nociplastic-like pain model in mice.

Profiling of LPS-induced acute systemic inflammation in the mouse.

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The lipopolysaccharide (LPS)-induced innate immune response in a mouse model follows fundamental principles crucial for understanding the host's defense mechanisms against bacterial infections. Reproducibility of the model is vital to ensure data are consistent and of high value when profiling novel drugs and modalities. Moreover, the ability to measure test drug levels in conjunction with cytokine levels in the same animal's plasma and tissues provides a valuable pharmacokinetic-pharmacodynamic relationship when investigating the *in vivo* properties of novel therapeutics. Our objective was to develop an LPS-induced innate immune response in a mouse model that serves as a valuable platform for the early development of novel therapeutic strategies in immune-related and chronic inflammatory diseases.

Our LPS mouse model involves dose response validation (0.3, 0,5, and 0.8 mg/kg LPS i.p.), drug comparators validation (PK/PD, e.g. TAK-242, Dexamethasone) as well as characterization of multiple organ inflammation such as brain, spinal cord, kidney, liver, and colon. Dexamethasone (5mg/kg s.c. or 10 mg/kg p.o.) was administered prior to LPS. Blood and tissue samples were collected at 4h post-model LPS induction. MSD assessed plasma and tissue cytokines (IL-6, IL-1β, TNF-α, IFN-γ). Test drug levels were quantitated using LC-MS.

LPS resulted in an increase of circulating and tissue IL-6, IL-1β, TNF-α, and IFN-γ vs. control. Dexamethasone, as well as TAK-242, significantly reduced their levels, $p<0.001$. Interestingly, Dexamethasone levels were 10x lower in the brain and 2x higher in the kidney than in the plasma, whilst TAK-242 levels were undetectable at termination time in both plasma and tissues.

Understanding LPS-induced responses combined with investigational drug pharmacokinetics enables us to profile a test drug's tissue distribution and efficacy in active inflammation. Importantly, the identification of anti-inflammatory drugs that can cross the blood-brain barrier (BBB) is vital for treating neuroinflammation and improving outcomes of patients with neurodegeneration.

Pudendal Nerve Constrictions (PNC) as a new animal model of vulvodynia

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Vulvodynia is a gynecological condition typified by persistent discomfort in the vulvar region. The enduring vulvar pain inherent to this disorder is linked with intense burning sensations, dyspareunia, and erythema. Current in vivo experimental models of vulvodynia, such as CFA-induced or candida albicans-induced vestibulodynia, only partially represent the total affected population. Indeed, among all, pudendal nerve entrapment is the most common causes identified to date and so, in our study, we investigated a novel in vivo model of vulvodynia obtained by the constriction of the pudendal nerve. Female C57BL/6J mice underwent constriction injury of the pudendal nerve. The vulvar mechanical allodynia was measured from day 1 post pudendal nerve constrictions (PNC) and data showed that surgery reduced the vulvar withdrawal threshold in mice compared to control group from day 3 to day 28 post PNC induction. At 21 days post-induction, we investigated the morphological changes as well as the proliferation of microglia cells in the dorsal horn of the spinal cord (L6, S1) through immunofluorescence approach. Morphological analysis of Iba-1 revealed an increased density of hypertrophic microglia in the spinal dorsal horn. Interestingly, the microglia reactivity was greater in the ipsilateral as compared to the contralateral dorsal horn, likely due to the surgery model. These preliminary data pave the way to conduct future behavioral, biochemical elctrophysiological and morphological studies in order to further characterize this new model. This model could represent a valuable tool for future preclinical research and drug discovery efforts.

Quantification and characterization of neutrophil-derived extracellular vesicles (EVs) using high-sensitivity flow cytometry.

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INTRODUCTION: Most viable cells generate EVs, which originate from their endosomal compartment (exosomes, 50-100 nm in size) and/or from plasma membrane blebbing and budding (ectosomes, 100-1000 nm). Neutrophil-derived ectosomes (NDEs) are produced in response to several stimuli and are involved in a myriad of physiological and pathological processes (1). Flow cytometry (FCM) is often used to study NDEs and other EVs. However, due to the small size of EVs, their measure is hindered by the unavoidable presence of non-vesicular nanoparticles in the samples, buffers, sheath fluid and other reagents, which can yield high background or even EV swarming by nonspecific events (2,3). These problems are fairly common and have been the subject of specialized publications (2-4). **METHODS:** Neutrophils were isolated from healthy donors and stimulated with fMLP to produce NDEs. These were isolated through serial centrifugations and analyzed by flow cytometry (Beckman Coulter Cytoflex) using the blue (SSC-488) and violet side scattering settings (SSC-405). The primary threshold was set on FSC (size) or calcein blue fluorescence, to compare the background signal. NDEs were characterized as events positive for Calcein Blue (closed vesicles), Annexin V (exposed phosphatidylserine) and expressing the neutrophil membrane markers CD15 or CD66b. This cytometry strategy was then applied to NDE quantitation in human plasma from healthy and septic non-fasting subjects. **RESULTS:** The use of SSC-405 increased the detection of EVs and other nanoparticles. However, event swarming and abort rate rose from <4% to 25-40%. The swarming of non-vesicular events could be mostly eliminated by changing the setting of the detection threshold from FSC (size) to calcein fluorescence. This reduced abort rates to <5% and yielded consistent results along a wide range of NDE concentrations. In plasma samples, NDEs were detected in low concentrations in healthy donors and were 10-fold more abundant in sepsis patients, without interference by lipoproteins or other analytes. **CONCLUSION:** We set up a highly sensitive, calcein-based cytometry method that eliminates most of the background noise by focusing on the acquisition of vesicular events without the interference of non-vesicular nanoparticles for NDE analysis in complex samples. Our method also dispenses with commonly used dilution and/or filtration steps during sample preparation.

Commented [PM1]: Let's not give away too much 😎

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WCI 2024 Abstract

Recommendations for Using Leptin and Adiponectin in Clinical Studies Evaluating Chronic Systemic Inflammation

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Leptin and adiponectin are adipokines that have been shown to mediate the relationship between systemic inflammation and chronic diseases. However, the inconsistent reporting of leptin and adiponectin data by previous studies hinders the pooling of data across studies for cross-population comparisons or meta-analyses. As such, the aims of this poster are to (1) encourage researchers in the area of systemic inflammation to include standardized measures of leptin and adiponectin and the leptin to adiponectin ratio in future studies, and (2) recommend standardized reporting methods. Following standardized measurement and reporting methods will increase the likelihood that new data generated can be compared across studies and thus, knowledge about the impact of these adipokines on inflammation regulation can be advanced.

Regulation of macrophage inflammatory response by potato-derived bioactive peptide

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The normal immune response to infection requires a well-regulated mechanism of leukocyte activation and recruitment, a process generally termed inflammation. Dysregulation of this inflammatory response is a major problem in modern medicine and is associated with several disease conditions including autoimmune diseases, cancer and even COVID-19.

Food-derived peptides with anti-inflammatory properties have gained popularity due to their availability in the daily diet and limited side effects. Previous reports have shown that the potato protein patatin has anti-inflammatory property. However, the sequence of amino acids in patatin that is critical for anti-inflammatory activity is not well established. In this work, we investigated the ability of the potato protein-derived peptide DIKTNKPVIF to down-regulate the inflammatory response of monocyte-derived macrophages (MDMs) activated with lipopolysaccharide (LPS). We found that DIKTNKPVIF inhibited the inflammatory response of MDMs to LPS as evidenced by decreased production of pro-inflammatory cytokines. Importantly, using computational and experimental screening techniques, we identified TNKPVI as the bioaccessible and biostable pharmacophore necessary for the activity of DIKTNKPVIF. Our results highlight the potential of potato-derived bioactive peptides to regulate the inflammatory response.

Resolvin E1-mediated stromal reprogramming via adaptive immunity enhances targeted cancer therapy

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While treatments targeting only tumor cells are not effective for many cancer types, tissue stroma as a critical driver of cancer offers an opportunity to target the tumor microenvironment. Cytotoxic chemotherapy and checkpoint inhibitors create tumor cell debris that may stimulate or induce tumor growth and metastasis. A paradigm shift is emerging in cancer therapy with the discovery of novel specialized pro-resolving mediators (SPMs), such as resolvin E1 (RvE1). However, no "pro-resolving" based therapy has been approved for cancer to date. Resolvin receptors are expressed by the major leukocyte types in the tumor stroma, i.e, macrophages, T lymphocytes, natural killer (NK), and dendritic cells. We hypothesize that RvE1, delivered as an active ingredient in a novel small molecule by subcutaneous injection, would reprogram leukocytes and enhance the clearance of cell debris. We demonstrate here that nanogram dosing of RvE1 (i.e. 30 ug/kg to 300 ug/kg) was efficacious when given QD, Q3D or Q6D and induced sustained tumor inhibition in pancreatic, lung, melanoma, and colon cancer in murine models. Remarkably, RvE1 enhanced immunotherapy (anti-PD1 or anti-CTLA-4) to transform a "cold" tumor into a "hot tumor" in pancreatic adenocarcinoma, Lewis lung carcinoma, and B16F10 melanoma. This suggests that RvE1 synergizes with chemotherapy and/or immune checkpoint inhibitors to potently inhibit tumor growth. We also evaluated RvE1 anti-tumor activity in the orthotopic KPC (KrasG12D/+/P53-/-/Pdx1-Cre) cell line, a robust model of human pancreatic ductal adenocarcinoma. In this model, RvE1 in combination with chemotherapy prolonged survival over 380 days, reduced tumor weight and prevented chemotherapy-induced metastasis. RNA-seq profiling revealed enrichment of CD8 T, dendritic, and NK cells, suggesting that RvE1 triggers an adaptive immune response. Depletion of CD8 T lymphocytes or NK cells abrogated the anti-tumor activity of RvE1. LC-MS-MS analysis revealed increased RvE1 concentrations in orthotopic pancreas tumors in treated mice compared to control. RvE1 also stimulated immune cell-mediated clearance of tumor debris. Taken together, this study introduces a novel molecular strategy for reprogramming tumor stroma via CD8 T lymphocyte and NK cell response. Thus, resolution-based SPM-directed stromal reprogramming with tumor-directed cytotoxic and immunologic drugs is a promising novel therapeutic approach for cancer.

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Reversing Microvascular Dysfunction in Heart Failure with Ejection Fraction > 40% using colchicine: The COL-Micro-HF Study Protocol

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Despite representing 50% of the heart failure (HF) population, clinical outcomes and quality of life of individuals with heart failure with mildly reduced (HFmrEF) or preserved (HFpEF) ejection fraction remain poor. Inflammation is central to the pathophysiological process of HF in many of these patients and is mainly driven by their numerous comorbidities. Many mechanisms have been postulated for this inflammatory milieu, amongst which myocardial damage due to coronary microvascular dysfunction (CMD) seems fundamental, reinforcing the importance of targeting inflammation as a potential treatment for patients with HFmrEF or HFpEF. Colchicine is an anti-inflammatory agent that has recently shown benefits in patients with coronary artery disease. In patients with HFmrEF or HFpEF, colchicine may improve CMD and circulating biomarkers of inflammation and fibrosis. In a single-center, randomized, double-blinded, placebo-controlled study, we aim to evaluate the effects of colchicine on CMD in patients with either HFmrEF or HFpEF and inflammation. We will enroll 70 participants with HF and left ventricular ejection fraction > 40% and elevated high-sensitivity C-reactive protein levels (hs-CRP, ≥2 mg/L). Patients will be randomly assigned in a 1:1 ratio to receive a 6-month course of colchicine at a dose of 0.5 mg twice daily or placebo. We will compare the change between baseline and six months in coronary flow reserve, a marker of CMD, assessed using adenosine-based positron emission tomography imaging. In addition, a broad set of circulating inflammation (including MPO, IL-1b, IL-6, TNF-a, sICAM-1) and myocardial fibrosis (including sST2, MMP-2/-9) biomarkers will be measured at baseline and six months. HFmrEF and HFpEF can be considered systemic inflammatory states induced by their frequent associated co-morbidities. While inflammation seems central to disease development and progression, whether a potent anti-inflammatory agent such as colchicine may improve CMD related to inflammation and circulating biomarkers of myocardial fibrosis is unknown. This mechanistic study aims to validate the role of inflammation as a potentially modifiable promoter of myocardial damage associated with CMD in patients with HFmrEF or HFpEF.

Senatore Cappelli wheat derived biomolecules exert anti-inflammatory and anti-oxidant activity

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Much attention has been focusing on wheat derived extracts for their health-promoting functions, as a source of bioactive molecules and nutraceuticals.

We evaluated in a multimethodological approach the anti-inflammatory and anti-oxidant properties of hydroalcoholic extracts obtained from husks, grains, flour and pasta derived from durum wheat belonging to the ancient cultivar "Senatore Cappelli", assessing their potential as bioactive compound sources in terms of phytochemical, antioxidant, and anti-inflammatory properties. The content of total phenolic compounds and total flavonoid and antioxidant activity were carried out by ABTS and DPPH assays. The cytotoxicity of the green extracts was evaluated by MTS and Trypan blue assays in the human U937 and murine microglia BV2 cells, showing that extracts do not affect microglia viability. We analyzed the inflammatory phenotype of cells pretreated by wheat extracts and then cultured in the presence or absence of LPS. We analyzed i) the expression of mRNAs for: IL-1 beta, IL-6, TNF-alpha, IL-17, IL-10, TGF-beta; ii) the expression of mRNAs of the main M1 and M2 polarization markers, including iNOS, ARG1, CD206, COX-2 and transcriptional factors TBX21, STAT1, STAT3, STAT4, STAT6, RORC, GATA3, FOXP3 and NR4A2; iii) the expression of anti-oxidant factors and enzymes (Nrf2, SOD1 and GPX). Extracts were shown to be devoid of any pro-inflammatory activity because they do not increase the expression of M1 markers. Conversely, expression of ARG-1, CD206, Chil3 mRNA was induced by the extracts also after the addition of LPS, reverting microglia toward an anti-inflammatory phenotype. Extracts of "Senatore Cappelli" wheat derivatives restore NRF2 expression related to the upregulation of SOD1, an antioxidant gene. It is known that NRF2 pathways counteract ROS production and inflammation in neurodegenerative disorders, suggesting that stimulation of NRF2 factor could play a key role as a therapeutic approach.

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Skin-Resident Macrophages and Microvesicles: Insights into the Immune Response of Wound Healing

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Macrophages, either resident or derived from blood monocytes, are key players in the wound healing process, with their differentiation influenced by the time elapsed since injury and the pathological or physiological state of the wound. While blood monocyte-derived macrophages have been extensively studied, skin-resident macrophages remain unexplored. In addition, extracellular vesicles (EVs), known for their involvement in intercellular communication and transport of bioactive elements, have a largely unknown effect on immune cells involved in wound healing. The content of EVs produced by myofibroblasts from healthy wounds has been previously highlighted, revealing the presence of pro- and anti-inflammatory cytokines. The aim of this study was to evaluate the role of EVs in the differentiation of skin-resident macrophages in normal and hypertrophic wound healing.

Dermal cells were isolated by a series of enzymatic steps and characterized by flow cytometry. The resulting cell mixture was cultured to induce macrophage differentiation into different subtypes: pro-inflammatory macrophages (M1) and anti-inflammatory macrophages (M2). Macrophages were then characterized by flow cytometry using specific markers. Fluorescent EVs were isolated from myofibroblasts of normal and hypertrophic scars.

In the dermal fraction, resident macrophages expressed the markers CD45 and CD163low. A protocol for differentiation of resident macrophages was developed and the differentiation state of macrophages was characterized by TNF-alpha expression for M1 and CD163high marker expression for M2. EVs will be added to undifferentiated macrophages and their differentiation state will be analyzed. We expect significant differences in macrophage behavior depending on the origin of the EVs used in co-culture, with a tendency for EVs from hypertrophic scars to promote differentiation into M2.

These findings will deepen our understanding of the role of EVs in the immune response associated with wound healing, opening new perspectives in the medical field.

Spatio-temporal Analysis of the TNF family cytokine RANKL in the immune and skeletal system

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Bone tissue is continuously remodeled through the concerted actions of bone cells, including osteoclasts and osteoblasts. An optimal balance between bone resorption by osteoclasts and bone formation by osteoblasts is required for bone homeostasis. The TNF family cytokine RANKL is an essential cytokine for osteoclast differentiaion. RANKL, which are produced by the supporting mesenchymal cells including osteoblast lineage cells binds to its receptor RANK on the precursor cells of monocyte/macrophage lineage. Pathologically, excess RANKL signal leads to abnormal osteoclast activation and bone loss in rheumatoid arthritis, osteoporosis, and bone metastasis. On the other hand, RANKL also plays crucial roles in the immune system, including lymph node development, thymic epithelial cell differentiation and M cell differentiation in the gut. Thus, RANKL exactly acts as a multifunctional cytokine that influences the skeletal and immune systems. RANKL is synthesized as a membrane-bound molecule, which is cleaved into the soluble form by proteases. By generating mice selectively lacking soluble RANKL, we have revealed that soluble RANKL is dispensable for physiological regulation of bone and immune systems. In addition, we found that soluble RANKL made no contribution to inflammation-induced bone desctrutsion. These findings indicated the importance of local regulation of the RANKL/RANK system. Since RANKL is a mulfuncaional cytokine produced in a wide variety of tissues, assessing the dynamics of the producing cells under both physiological and pathological conditions is important for the elucidation of the pathogenesis of various bone loss diorders. We established the strategies to analyze the spaito-temporal expression of RANKL, which would contribute to the understanding of the mechanisms underlying the local regulation of the RANKL/ RANK system and the development of the therapeutic intervention for various bone loss.

Spinal and supraspinal characterization of an inflammatory CFA-induced model of Vulvodynia

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Vestibulodynia is a complex pain disorder characterized by chronic discomfort in the vulvar region, often accompanied by tactile allodynia and spontaneous pain. In patients a depressive behaviour is also observed. In this study, we have used a model of vestibulodynia induced by complete Freund's adjuvant (CFA) in female C57BL/6J mice focusing our investigation on the spinal cord neurons and microglia. We investigated tactile allodynia, spontaneous pain, and depressive-like behavior as key behavioral markers of vestibulodynia. In addition, we conducted in vivo electrophysiological recordings to provide, for the first time to our knowledge, the characterization of the spinal sacral neuronal activity in the L6-S1 dorsal horn of the spinal cord. Furthermore, we examined microglia activation in the L6-S1 dorsal horn using immunofluorescence, unveiling hypertrophic phenotypes indicative of neuroinflammation in the spinal cord. This represents a novel insight into the role of microglia in vestibulodynia pathology. To address the therapeutic aspect, we employed pharmacological interventions using GABApentin, amitriptyline, and PeaPol, a combination of palmitoylethanolamide and polydatin. Remarkably, all three drugs, also used in clinic, showed efficacy in alleviating tactile allodynia and depressive-like behavior. Concurrently, we also observed a normalization of the altered neuronal firing and a reduction of microglia hypertrophic phenotypes.

Stratification of Rheumatoid Arthritis Patients and Assessment of Molecular Targeted Therapies' Efficacy

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[Objective] Rheumatoid arthritis is a prototypical inflammatory disease characterized by persistent chronic inflammation. Our study focused on immunophenotypic stratification, aiming to optimize molecular targeted therapies for rheumatoid arthritis.

[Methods] We stratified 533 bio-naive patients diagnosed with rheumatoid arthritis through immunophenotyping of immunocompetent cells (T cells, B cells, NK cells, monocytes, and dendritic cells) in peripheral blood using flow cytometry. We analyzed the treatment response using molecular targeted drugs, ensuring matching of patient backgrounds through inverse probability of treatment weighting (IPTW).

[Results] Cluster analysis stratified 533 RA patients into 5 clusters. 2 of these showed distinctive RA phenotypes differing HC, marked by significant increases in CD4 effector memory T cells re-expressing CD45RA (TEMRA). We assessed the clinical efficacy of each molecular targeted therapy, including TNF inhibitor, IL-6 inhibitor, CTLA4-Ig, and JAK inhibitor, within each group and observed significant differences in their effectiveness. Subsequently, within each cluster, the utilization of b/tsDMARDs with high efficacy was designated as the Preferred group, while the use of alternative drugs was categorized as the Non-preferred group. In the Preferred group, both the 24-week remission rate and the rate of achieving low disease activity were significantly higher compared to the Non-preferred group. To validate these findings, immunophenotyping was conducted in a new validation cohort comprising 183 cases. The subjects were then reassigned to the aforementioned clusters using the k-nearest neighbor method. Significantly, in the validation cohort, the remission rate of the Preferred group at 24 weeks exceeded that of the Non-preferred group, demonstrating an effectiveness more than twice as high.

[Conclusion] Immunophenotypic stratification underscored the potential for treatment optimization.

Synthesis of Anti-Inflammatory Compounds Targeting TNFa and IL-1B Signaling Pathways in the Treatment of Traumatic Brain Injury

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Traumatic Brain Injury (TBI) represents a significant global health concern, contributing to long term disability and cognitive impairments. TBI induces a robust inflammatory response with levels of cytokines such as TNFα and IL-1B, playing pivotal roles in the secondary injury cascade. Synthesized molecules will be discussed and if they have a potential neuroprotective effect on inhibiting TNFα and IL-1B and thus reducing neuronal cell death, preserving synaptic integrity and enhancing neuroregeneration.

Synthesis, biosynthesis and molecular targets of the monoacylglycerol and the *N*-acyl-ethanolamine of stearidonic acid

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INTRODUCTION. Diets enriched in omega-3 polyunsaturated fatty acids fish oils have been linked with beneficial anti-inflammatory effects. However, their daily consumption is not palatable to many, due to their fishy flavour. Additionally, questions regarding the sustainability of fish oils support the exploration of alternative sources. These include plant-derived oils like the stearidonic acid (SDA)-rich oil from *Buglossoides arvensis* (ahiflower) seeds, which has been shown to be more effective than Flax seed oil at enriching tissues with long-chain omega-3 polyunsaturated fatty acids. We recently documented that unsaturated fatty acids can be acylated into glycerophospholipid and remodeled into monoacylglycerols and *N*-acyl-ethanolamines in human leukocytes. In order to better understand the putative beneficial effects of a SDA-enriched diet and the cellular/molecular mechanisms involved, we postulated that, and investigated if, SDA also undergoes such metabolism, possibly yielding undocumented bioactive effectors.

METHOD. We chemoenzymatically synthesized 1-stearidonoyl-glycerol (SDG) and *N*-stearidonoyl-ethanolamine (SDEA). We optimized their detection/quantification by tandem mass spectrometry and assessed their functional impact in different models.

RESULTS. In line with out previous data with several PUFAs, SDA-treated neutrophils biosynthesized a significant amount of 1/2-SDG as well as a limited amount of SDEA. This was concentration-dependent and the levels of 1/2-SDG obtained were in between those obtained for 2-arachidonoyl-glycerol and 1/2-linoleoyl-glycerol when cells were treated with their respective precursors, arachidonic acid and linoleic acid. In contrast to 2-arachidonoyl-glycerol and *N*-arachidonoyl-ethanolamine, neither 1-SDG nor SDEA were ligands for the cannabinoid receptors 1 and 2. However, 1-SDG activated both PPARα and PPARγ while SDEA selectively activated PPARα. Of note, neither the monoacylgycerol nor the *N*-acyl-ethanolamine of linoleic acid had an impact on these transcription factors. Finally, an ahiflower oil-enriched diet led to an increase in 1/2-SDG levels in the joints of arthritic mice (K/BxN model), which coincided with a decrease in inflammatory score.

CONCLUSIONS. 1) Human neutrophils biosynthesize the SDA metabolites 1/2-SDG and SDEA. **2)** 1/2-SDG activates both PPARα and PPARγ while SDEA only activates PPARα. **3)** Neither 1-SDG nor SDEA bound to the cannabinoid 1 or 2 receptors. **4)** An SDA-enriched diet diminishes the severity of experimental arthritis. While the cellular/molecular mechanisms involved in the beneficial effect of SDA and its metabolites remain to be solved in arthritic mice, their impact on PPAR(s) might explain, at least in part, the beneficial effect of this PUFA in experimental arthritis and other inflammatory conditions. We are currently exploring this possibility.

Targeting S1PR1 for Post-acute Neurocognitive Sequalae of SARS-CoV-2

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Cognitive impairment is one of the most widely reported neurological symptoms of the post-acute sequalae of SARS-CoV-2 infection. There are no FDA-approved drugs to treat this condition. The SARS-CoV-2 spike protein attaches to angiotensin converting enzyme 2 (ACE2), initiating ACE2 internalization for infection, decreasing membrane-bound ACE2 and shifting the renin angiotensin system towards Ang II production. By activating the Ang II type 1 receptor (AT_1R), Ang II can induce neuroinflammation and oxidative stress that contribute to cognitive impairment in several animal models (e.g., heart failure, AD). Therapeutics targeting Ang II/AT_1R are protective against cognitive impairment in human and animal studies. Ang II also alters sphingolipid metabolism by activating enzymes involved in the biosynthesis of sphingosine-1-phosphate (S1P), a potent inflammatory sphingolipid which we have implicated in the development of cognitive impairment in a model of neurotoxicity. Noteworthy, studies of COVID-19 patient serum have shown increased levels of S1P predicting severity. We developed a mouse model that blocks ACE2 with chronic delivery of the ACE2 inhibitor MLN 4760 (ACE2i model) which results in impairment in the spatial memory and recognition memory mimicking the cognitive deficits associated with SARS-CoV-2. Our data revealed increased S1P levels in the hippocampus and prefrontal cortex (PFC), key centers of cognition, where we found S1PR1 expression. This was associated with markers of oxidative stress and neuroinflammation (two proposed mechanisms thought to drive cognitive deficits in various animal models). Systemic administration of the orally bioavailable functional S1PR1 antagonist ozanimod prevented oxidative stress, neuroinflammation and cognitive impairment in the ACE2i model in the same behavioral tests without affecting locomotor activity. Our results suggest targeting the S1PR1 receptor with ozanimod can prevent the cognitive impairment that occurs from SARS-CoV-2 infection.

The immune regulator IRL201805 alters T regulatory cell phenotype in gut biopsies *ex vivo* from inflammatory bowel disease patients

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Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis, both are characterized by chronic inflammation and damage to the GI tract. There is no cure for IBD and the lack of effective long-term treatments means patients may eventually undergo surgery to remove damaged portions of the GI tract. IBD is caused by release of inflammatory proteins from bacteria, blood or gut resident T-cells. Regulatory T-cells (Tregs) are known to be important in preventing IBD. Treg surface activation receptor CD69 and the ecto-ATPase - CD39 (that metabolises inflammatory ATP) in part mediate immunosuppression. Immune cells from the lamina propria isolated from gut biopsy tissue, from treatment naive patients with IBD and non-IBD controls, was incubated with IRL201805 overnight. Mononuclear cells were then stained with a 24-antibody panel and analysed by spectral flow cytometry. IRL201805 increased the generation of double positive CD69+/CD39+ FoxP3 Tregs in inflamed but not non-inflamed tissue. In separate monocyte cultures we observed IRL201805 induced the increase in the cell surface ligands for CD69, galectin-1, and S100A8/9 as detected by quantitative Tandom Mass Tag™ spectrometry. In monocyte-T cell co-cultures IRL201805 led to an increase in STAT5 phosphorylation, which is known to promote Treg differentiation and prevent pro-inflammatory Th1/Th7 cell generation. Therapeutics able to enhance CD69+/CD39+ Tregs in intestinal tissues *in vivo*, may play an important role in immune system balance and the prevention of inflammation.

Keywords:
Inflammatory bowel disease, gut biopsies, IRL201805, T regulatory cells, spectral flow cytometry.

The Immunometabolic Bimodal Mechanism of NLRX1 Agonist NX-13 in a Pig Model of Ulcerative Colitis

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In mouse models, NX-13 is an orally active, gut-restricted NLRX1 agonist that reduces colitis in multiple UC models through a novel immunometabolic, bimodal mechanism. Most preclinical UC studies rely upon rodent models, though the human gastrointestinal physiology, microbiome, and immune system bear a greater similarity to that of pigs. Here, we validate our preclinical murine NX-13 results using the dextran sulfate sodium (DSS) pig model of colitis and confirm the immunometabolic mechanism of action. Pigs were randomized by weight (n=6 per group) and were administered tablets of placebo, NX-13 10 mg, NX-13 50 mg, or NX-13 100mg once daily. Simultaneously, pigs were challenged with 1% DSS in drinking water for 6 days and monitored daily. Feces were collected for fecal calprotectin (FCP) quantification. Necropsy was conducted on day 7, and colonic tissue was macroscopically scored and collected for analysis by flow cytometry, histopathology and gene expression. Oral NX-13 treatment protected against weight loss and reduced colitis development with differences as early as day 2, becoming significant between days 4 and 6 (Fig1A). NX-13 yielded a dose-dependent improvement in macroscopic lesion severity and microscopic immune cell infiltration. Specifically, Th1 cells (Fig1B) and TNF producing myeloid cells in the colonic were reduced by NX-13, as well as FCP levels in stool (Fig1C) and mRNA expression in the colon. Oral NX-13 treatment reduced inflammatory cytokines and chemokines. Additionally, NX-13 treatment had MOA specific effects, namely upregulation of NLRX1 (Fig1D) and mitochondrial metabolism gene COX3, and reduction of NFkB and NLRP3. NX-13 demonstrated fast and clinically meaningful improvement in disease activity and impact on key inflammatory markers. These results can be used to guide translational studies in the ongoing NEXUS phase 2 clinical trial.

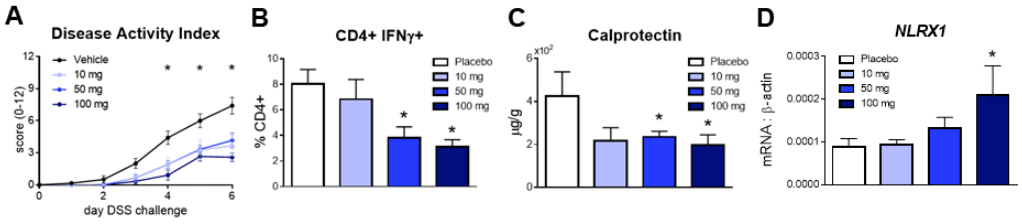

Figure 1: Oral dosing of NX-13 tablets in pigs with acute DSS colitis reduced over all disease severity (A), colonic Th1 effector population (B), fecal calprotectin in stool (C),and mRNA expression of *NLRX1* (D). Data are presented as mean ± SEM. Quantitative data were analyzed using ANOVA,*p<0.05

The immunomodulatory activity of *Mangifera indica* L. Extract (MIE) in inflammatory bowel disease (IBD)

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Inflammatory bowel disease (IBD) is defined by chronic intestinal inflammation, resulting from perturbation of the intestinal barrier. Disease pathology is driven primarily by a repertoire of T cells including T helper (Th) 1, 17 and T regulatory (Treg) subsets. A considerable number of patients are non-responsive to conventional treatments with biologics and immunomodulators calling for a need for new therapeutic strategies. Growing evidence demonstrates that dietary polyphenols derived from Mangifera indica L. extract (MIE), has the potential to alleviate intestinal inflammation and modulate T cell levels in spleen. This study explores the anti-inflammatory and immunomodulatory properties of MIE by utilising blood from adult IBD patients followed by a pre-clinical model of T-cell driven colitis.

Clinically, MIE demonstrated a significant reduction in TNFα levels in an ex-vivo model of intestinal barrier breakdown using LPS-spiked blood from IBD patients. Further investigation in a model of T cell driven colitis revealed a significant improvement in clinical severity with MIE treatment, marked by improved weight loss and levels of faecal calprotectin. This was accompanied by repaired intestinal barrier permeability via restoration of intestinal metabolites and levels of epithelial cell junctional proteins, ZO-1 and occludins. Pathogenic infiltration of T cell subsets in colonic tissue was also reversed by MIE, characterised by the reduced frequency of Th1 and Th17 cells and increase in Treg cells observed via flow cytometry analysis. Additional mechanistic insights uncover direct effects of MIE on vascular endothelium, inhibiting TNFα and IFNγ-induced upregulation of COX and DP2 receptors that contribute to lymphocyte transmigration. Collectively, this study demonstrates the therapeutic benefits of MIE in restoring immunological imbalance during the onset of colitis and its potential use to treat IBD in clinic.

The Iron Chelator, DIBI, Reduces Bacterial Load and Inflammation in Experimental Lung Infection

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Iron plays a critical role in lung infections due to its function in the inflammatory immune response but also as an important factor for bacterial growth. Iron chelation represents a potential pharmacological approach to inhibit bacterial growth and pathologically increased pro-inflammatory mediator production. The present study was designed to investigate the impact of the novel iron chelator, DIBI, in murine lung infection induced by intratracheal *Pseudomonas aeruginosa* (strain PA14) administration. DIBI (80mg/kg) was given by intraperitoneal injection either as a single dose immediately after PA14 administration or a double dose (second dose 4 h after PA14 administration). The results showed that lung NF-κB p65 levels, as well as levels of various inflammatory cytokines (IL-6, TNF-α, IL-1β) both in lung tissue and bronchial alveolar lavage fluid (BALF), were significantly increased 24 h after PA14 administration. Single-dose DIBI did not reduce the inflammatory response and bacterial load in the lungs or BALF. However, two doses of DIBI significantly attenuated levels of NF-κB p65, reduced levels of inflammatory cytokines, and decreased bacterial load. Our findings support the conclusion that the iron chelator, DIBI, can reduce bacterial growth and exhibits anti-inflammatory effects in experimental lung infection induced by *P. aeruginosa*.

The Placental Growth Factor: A key player in Skin Fibrosis of Systemic Sclerosis and Hypertrophic Scars?

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Systemic Sclerosis (SSc) is a rare systemic autoimmune disease. Hypertrophic scars (Hsc) occur after deep skin injuries. Their common hallmark is excessive pathological skin fibrosis due to extracellular matrix (ECM) accumulation. Current treatments didn't delay or stop fibrosis progression, highlighting the urgent need for novel therapeutic strategies. In this context, we dug into the role of the Placental Growth Factor (PlGF) in skin fibrosis development.

Fibroblasts were isolated from skin biopsies of healthy individuals, SSc or Hsc patients, and cultured in monolayer or three-dimensional (3D) to form a reconstructed dermis. PlGF expression was visualized in skin biopsies and 3D reconstructed dermis using immunofluorescence. PlGF effect on the ECM was assessed using fluoroenzymatic and enzyme-linked immunosorbent assay in fibroblast monolayers or reconstructed dermis following treatments with PlGF-1 or -2, VEGF or TGF-β. Finally, the dermal thickness of the 3D reconstructed dermis, with or without treatments, was measured with a laser telemeter.

PlGF was absent in healthy skin but expressed in Hsc skin and 3D reconstructed dermis from Hsc or SSc fibroblasts. PlGF treatment stimulated collagen I production in healthy fibroblasts cultured in monolayer and 3D reconstructed dermis from healthy and Hsc fibroblasts. Moreover, PlGF treatment increased dermal thickness in 3D reconstructed dermis from healthy and SSc fibroblasts.

PlGF may directly contribute to fibrosis development by promoting ECM production. Targeting PlGF could represent a new therapeutic approach in skin fibrosis associated with SSc or Hsc, with potential applications in other fibrosis-related diseases.

The role of Formyl Peptide Receptor 2 in inflammatory arthritis

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Acute inflammation is a protective mechanism of our body against pathogens or tissue damage, which actively resolves to return the inflamed tissue to homeostasis via the production of endogenous pro-resolving mediators. However, it is now thought that chronic inflammatory diseases such as arthritis may persist due to a failure of resolution responses. Therefore, new therapeutic approaches that promote the resolution of inflammation may offer a useful strategy to tackle the disease burden of Rheumatoid arthritis. The Formyl Peptide Receptor 2 (FPR2, also known as ALX) is a G protein-coupled receptor (GPCR) that promotes the resolution of inflammation through binding of several naturally occurring agonists including small bioactive lipids such as specialized pro-resolving mediators e.g. lipoxin A4 and resolvin D1 and proteins such as annexin A1. In fact, previous studies have demonstrated that mice lacking the *Fpr2* gene in all cells and tissues (global Fpr2 knockout mice) present a more exacerbated and prolonged response to K/BxN serum transfer induced arthritis (STIA). Herein, we utilized a humanized FPR2 (hFPR2) mouse colony, bearing an intact or a selective receptor deficiency in myeloid cells (LysM-cre) to dwell on the cellular mechanisms. These mice were characterized by the detection of hFPR2-GFP in whole blood, whereby the GFP (hFPR2) expression in circulating neutrophils was reduced to 30%. hFPR2 flox mice and myeloid cell-specific hFPR2 KO mice were subjected to STIA. Whilst the clinical score was similar between genotypes at day 6 of arthritis, the LysMcre FPR2 KO showed an increased number of neutrophils (Ly6G+) within the arthritic joints at peak disease. Interestingly, whilst hFPR2 expression was absent on joint infiltrating neutrophils, expression on resident macrophages was significantly increased. Further studies investigating whether the resolution phase of arthritis is prolonged in these mice is currently being performed. Taken together, these findings provide further evidence of the protective role of FPR2 in limiting myeloid cell infiltration to the joint during arthritis.

The role of interleukin-1 receptor isoforms in inflammation and pain

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INTRODUCTION: Pain affects 20% of adults worldwide. In patients with inflammatory autoimmune diseases such as multiple sclerosis (MS), this prevalence is over 50%. Painful information is transmitted from the periphery to the spinal cord and then the brain through the dorsal root ganglia (DRGs), where sensory neurons called nociceptors reside. Interleukin-1β (IL-1β) is a pro-inflammatory cytokine capable of triggering both inflammation and pain. OBJECTIVE: To identify the mechanisms mediating inflammatory and painful responses induced by IL-1β. METHODS: IL-1β injections were performed in the cerebellomedullary cistern of wild-type mice, mice globally knockout for the *Il1r1* gene, and mice in which *Il1r1* gene expression was specifically knockdown or restored in TRPV1+ nociceptors. We then characterized neurons expressing IL-1R1, studied the presence of transcription starting sites (TSS) from total RNA extracted from DRGs using 5'RACE-PCR, and performed immunoblotting to verify the presence of IL-1R1 isoforms. We also studied the expression of the two isoforms of the IL-1R1 coreceptor, IL-1RAcP and IL-1RAcPb, by *in situ* hybridization. RESULTS: IL-1R1 is expressed exclusively in a subtype of nociceptors expressing TRPV1. Invalidation of the *Il1r1* gene in these nociceptors prevented tactile pain (allodynia) in mice with experimental autoimmune encephalomyelitis, a model of MS, without affecting other clinical signs. Restoration of IL-1R1 resulted in the return of painful behaviors. We also identified a TSS encoding a novel *Il1r1* mRNA and confirmed the existence of a novel truncated isoform that we named τIL-1R1. Finally, we detected the presence of IL-1RAcP and IL-1RAcPb in DRG neurons. CONCLUSION: IL-1β triggers inflammation and pain through independent mechanisms. Painful response is likely to be mediated by TRPV1+ nociceptors via the τIL-1R1 isoform.

To Resolve Or Not To Resolve: The Role Of FPR2 Receptor During Coronavirus Infection

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Resolution of inflammation is a crucial process for restoring tissue homeostasis following an injury. Formyl peptide receptor 2 (FPR2) is a G-protein coupled receptor (GPCR) that plays a fundamental role in the resolution of inflammation by binding to various pro-resolving molecules. Misplaced inflammation is a major contributor to tissue damage and mortality associated to various infectious agents, such as SARS-CoV-2. Here, we assessed the role of the FPR2 receptor during Betacoronavirus infection. Wild-type (WT) mice and FPR2/3 knock-out mice (FPR2/3KO) were intranasally infected with a strain of murine betacoronavirus (MHV-3), which mimics severe COVID-19, by causing pneumonia and death in mice. FPR2/3KO mice showed approximately 50% protection from lethality when exposed to a lethal MHV-3 inoculum, compared to WT mice, in which 100% succumbed to infection by days 5-7. Interestingly, a logarithmic reduction in MHV-3 inoculum resulted in 100% protection against lethality in FPR2/3KO mice, while all WT mice were deceased by day 6-10 post-infection. FPR2/3KO mice also exhibited reduced viral titers in the lungs, liver, plasma and spleen, decreased lung damage and diminished production of inflammatory mediators. FPR2/3KO mice also displayed higher numbers of eosinophils in the lungs and increased expression of eosinophil peroxidase (EPO) when compared to WT mice. Mechanistically, eosinophils, alveolar macrophages and activated dendritic cells of FPR2/3KO mice exhibit increased expression of iNOS as evaluated by flow cytometry. Pharmacological blockade of FPR2 using a selective inhibitor WRW4 (8mg/kg, intraperitoneal route, daily) resulted in reduced systemic viral titers in the spleen without exerting any additional protective effects on the lungs. Surprisingly, these findings suggest that inhibition of FPR2/3 receptors could represent a promising therapeutic target against Betacoronavirus infection.

Unraveling mechanisms of macrophage extracellular trap (MET) formation: a role of reactive nitrogen species (RNS)

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Extracellular trap (ET) formation, initially recognized in neutrophils, has emerged as a phenomenon exhibited by various innate immune cells, including monocytes/macrophages. Monocyte/macrophage extracellular traps (METs) constitute a novel strategy in the cellular defense repertoire against pathogens. Despite their significance, the mechanistic insights into MET formation remain incomplete. In this study, we aimed to validate the formation of METs by bone marrow-derived macrophages (BMDMs) and to delineate the involvement of reactive nitrogen species (RNS) in this process. BMDMs were generated by differentiation of bone marrow cells from C57BL/6J male mice. Differentiation was facilitated using conditioned medium from the mouse fibroblast cell line L929, and functional activity was confirmed by flow cytometry, assessing the expression of $F4/80^+$ and $CD11a^+$ markers. Differentiated BMDMs were subjected to stimulation with a nitric oxide synthase (NOS) inhibitor (L-NAME), a nitric oxide donor (SNAP), or lipopolysaccharide (LPS). Visualization of METs was achieved through confocal microscopy, employing Sytox Green DNA stain and antibodies targeting MET components, including histones (H2A.X) and MMP-9. Our findings demonstrate that fully differentiated and functional macrophages, characterized by adherence, morphology, iNOS expression, and NO production, are proficient in forming METs upon stimulation. Notably, we present evidence for the pivotal role of RNS in MET release. L-NAME effectively inhibited MET formation by BMDMs in response to LPS, while SNAP induced METs. This study unveils the intricate interplay between macrophages, reactive nitrogen species, and MET formation, shedding light on a previously unexplored aspect of macrophage-mediated immune responses. The study was funded by the National Science Centre of Poland (grant 2021/43/B/NZ6/00782).

Vaccine or Season? The Impact of SARS-CoV-2 Vaccination and Seasonal Variation on the Inflammatory Response of Neutrophils

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The approval of vaccines against the SARS-CoV-2 virus placed much hope in the attenuation of the global cost of the pandemic. As the innate immune response is an important first checkpoint in the evolution of an infection, we sought to determine the impact of vaccination on neutrophil activation by a viral analog acting on TLR 7/8 − R848. To this end, a cohort of 304 food and retail workers from Quebec City have given blood samples for three visits at 12-week intervals. Neutrophils were isolated at the first and third visits and were directly stimulated with R848 to assess the innate immune response. After 24h, supernatants were collected to measure IL-8 production by ELISA. In view of the study's scope, from Spring 2021 to Spring 2022, we compared neutrophil response throughout the seasons. The results showed that IL-8 production after stimulation was significantly different in our cohort depending on the season of the visit. This difference was observed in vaccinated and unvaccinated participants. We also found that the participants' response changed between visits, and that there was an increase in IL-8 production after vaccination. More research is needed to understand the connection between vaccination and season on the neutrophil. Another finding was that subjects who received the Moderna vaccine produced more IL-8 at resting than the unvaccinated and Pfizer groups, and that resting levels of IL-8 were lower for subjects who received their last vaccine dose more than 4 months prior to the visit. This study highlights that neutrophil response could be affected by the season, and by vaccination. It also shows the importance of taking into consideration the period of sampling when studying neutrophils and innate immunity.