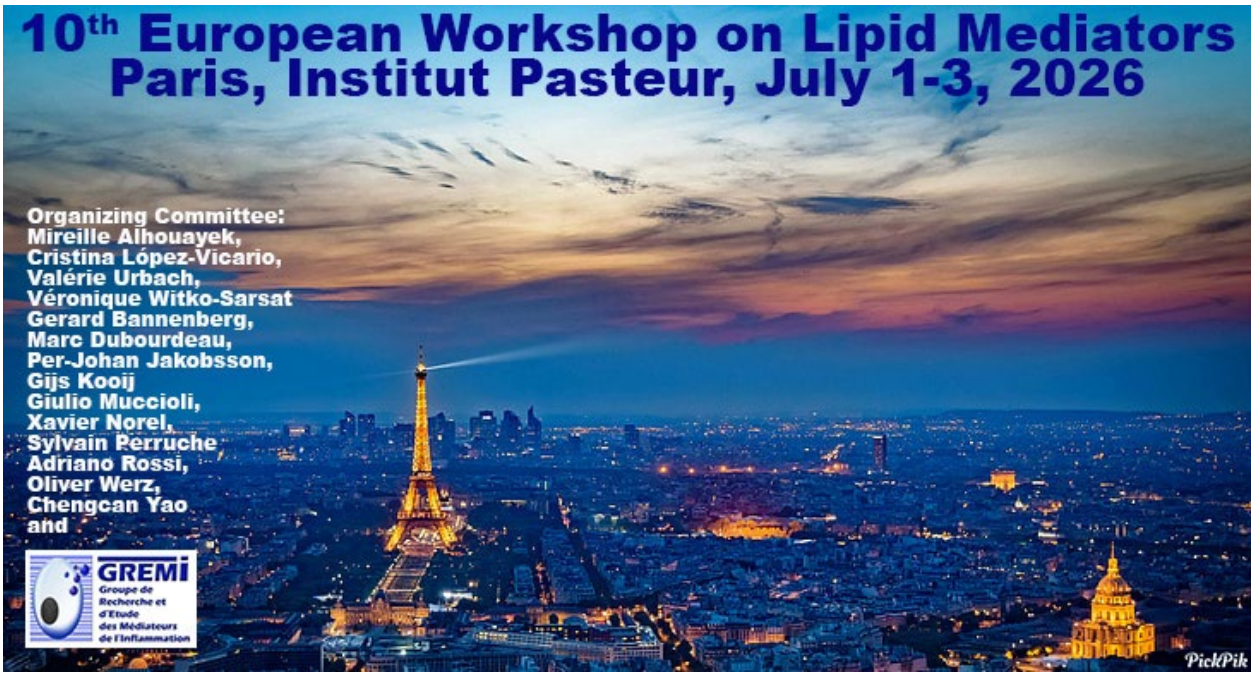

BOOK OF ABSTRACTS

10th European Workshop on Lipid Mediators

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ORAL PRESENTATIONS

Educational Session

HOW TO ANALYSE NEO-PUFAS AND NATURAL LIPOPHENOLS: THANKS TO LC-MS/MS AND TARGETED LIPIDOMICS

Thierry Durand

Institut des Biomolécules Max Mousseron, UMR 5247, CNRS, UM, ENSCM, Département Lipides, Pôle Chimie Balard Recherche, 1919 route de Mende, 34000 Montpellier, France

Oxidative stress plays a crucial role in various pathological conditions, making lipid oxidation products important mediators of cellular responses. 4(*RS*)-4-F_{4t}-neuroprostane (4-F_{4t}-NeuroP), non-enzymatic oxygenated metabolites (NEO-PUFA)[1] of one of the major PUFA in the brain (DHA, C22:6 n-3), has been recognised for its diverse beneficial biological properties. Our transdisciplinary research has identified its anti-arrhythmic properties[2], potential anti-apoptotic activity[3] and neuroprotection on microglia[4]. Furthermore, 4-F_{4t}-NeuroP has been shown to possess anti-inflammatory effects in macrophages[5], and targeted VIDD (Ventilator-Induced Diaphragm Disfunction)[6].

Lipophenols, polyphenolic compounds acylated by a fatty acid, have recently been identified[7] in food matrices naturally rich in both polyphenols and fatty acids, making them natural derivatives present in human diet. The identification of natural lipophenols is particularly relevant to understand their pharmacological actions, metabolism or to use them as analytical standards.

The lecture will present how to quantify NEO-PUFAs and Natural Lipophenols by LC-MS/MS, targeted lipidomics, in different matrices (human, animals, plants, seeds…)[7,8,9].

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RNA-BASED CONTROL AT THE SITE OF SIGNAL GENERATION

Beatrix Suess

Department of Biology, TU Darmstadt, Germany

The importance of RNA has grown enormously, and naturally it also plays an important role in the regulation of lipid mediators. I will present an overview of the various levels of RNA-mediated regulation, explain the underlying mechanisms, and present examples of how the synthesis of lipid mediators can be controlled at the RNA level. I addition, I will address how this control can be conditionally regulated using synthetic RNA switches.

MicroRNA REGULATION AT THE ORIGIN OF LIPID MEDIATOR SYNTHESIS

Dieter Steinhilber

Goethe University Frankfurt, Germany

Lipid mediators are considered to control many physiological and immunological functions. The biosynthesis and signalling of lipid mediators such as eicosanoids or other oxylipins derived from eicosapentaenoic or docosahexaenoic acid depends on the expression of the oxylipin forming enzymes such as lipoxygenases and cyclooxygenases and the corresponding oxylipin receptors which are required for oxylipin signal transduction. The function of these enzymes and receptors is often fine-tuned by microRNAs which regulate the expression of the respective mRNAs as well as their translation into functional proteins. The lecture will address examples of the regulation of enzymes of the eicosanoid pathways by microRNAs as well as the regulation of Dicer activity by 5-lipoxygenase.

1st Sponsored Session

4 D LIPIDOMICS FOR CELLULAR AND CIRCULATORY PHENOTYPING WITH DISEASES

Laura Bindila

Clinical Lipidomics Unit, Institute of Physiological Chemistry, University Medical Center of Mainz, Germany

4 Dimensional (4D) - Lipidomics is increasingly emerging as a transformative lipid research technology in health and disease. With the added capability of ion mobility mass spectrometry, lipid species, including conformational and compositional isomers can be better resolved, detected and identified advancing the lipidome coverage in various biological matrices. 4D- Lipidomics with trapped ion mobility mass spectrometry (TIMS) has been recently introduced and developed into high-throughput routine profiling technology for large clinical cohorts profiling, including neurodegenerative, cardiovascular diseases and healthy aging studies. Concurrently, cellular lipidomics profiling using 4D-TIMS platform integrated with transcriptomics analysis was expedited for low number single cell populations for cellular phenotyping in cellular and animal models of immune and cardiovascular diseases. A 4D-TIMS pipeline has been developed and advanced for profiling immuno lipids carrying neuro immuno epitopes in Parkinson diseases. The 4D-TIMS Lipidomics portfolio along with their successful application for cellular and circulatory phenotyping in health and diseases will be presented herewith.

SPECIES-LEVEL BIOMARKERS OF MASLD AND MASH PROGRESSION IN NORMOLIPIDEMIC INDIVIDUALS WITH OBESITY

Negar Mir, Juan JA Henao, André Tchernof, Anthony J Hanley, Jacqueline L Beaudry, Adam H Metherel

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Justification: Metabolic dysfunction–associated steatotic liver disease (MASLD) and metabolic dysfunction–associated steatohepatitis (MASH) are associated with dyslipidemia. However, the current diagnostic methods fail to predict the disease in the early stages and the gold standard method (liver biopsy) is invasive. This provides an interest in plasma lipidomics as a minimally invasive approach to identify disease-associated lipid species. Objective: To apply untargeted lipidomics to identify specific plasma or liver lipid species candidates that are associated with disease progression in MASLD and/or MASH. Methods: Plasma and liver biopsies from 30 normolipidemic individuals (mean age: 47 ± 7 years) with obesity (mean BMI: 46.5 ± 7.4 kg/m2) who underwent bariatric surgery were divided into 3 groups based on liver health (Healthy, MASLD, and MASLD+MASH, n=10 per group). Lipids were extracted and analyzed via LC-MS/MS in both negative and positive ionization mode. Data processing and lipid identification was performed using LipidMatch suite 5.4 and peak areas normalized to class-specific deuterated internal standards (SPLASH LipidoMix). Statistical analysis included one-way ANOVA and Fisher's LSD post-hoc test. Heatmaps of the top 25 plasma and top 50 liver ANOVA-selected lipid species were chosen to evaluate disease-related lipid-specific clustering. Additional analysis will be performed to investigate lipid species changes based on percent steatosis, and largest absolute changes. Results: Heatmaps of the top species for both liver and plasma revealed a stable triglyceride-rich (TG-rich) versus TG-poor clustering pattern, confirming that the dominant signal was robust and not an artifact of selecting a very small feature list. Post-hoc results showed that many of the top hits were driven by MASLD versus Healthy and MASLD+MASH comparisons. Specific TG species, particularly TG (12:0_18:1_18:3) and TG (18:0_18:1_22:4) were found to be elevated in plasma of MASLD patients, compared to healthy and MASLD+MASH groups. Significance: These findings highlight the limitations of broad class-based analysis and underscore the importance of species-level resolution. Even though the total circulating TGs were normal in this population, the accumulation of specific plasma TGs in MASLD could be used as a potential biomarker of the transition to MASLD in normolipidemic individuals and may be predictive of liver TG accumulation.

PROFILE AND ORIGIN OF LIPID MEDIATORS IN PNEUMOCOCCAL MENINGITIS

Serhii Chornyi[1,2], Nora Chekrouni[3], Marian A. van Roon[3], Nico Hahn[1], Marieke Heijink[2], Jan Van den Bossche[1], Martin Giera[2], Matthijs C. Brouwer[3], Gijs Kooij[1], Diederik van de Beek[3]

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Bacterial meningitis is a life-threatening infection of the central nervous system. *Streptococcus pneumoniae* is the most common causative pathogen and is associated with high mortality and a high rate of neurological sequelae. Unfavorable outcomes in pneumococcal meningitis are associated with a dysregulated host inflammatory response. Lipid mediators (LMs) are key regulators of inflammation and likely contribute to the inflammatory response during meningitis, but their presence, origin, and function in pneumococcal meningitis have not been fully characterized. We performed liquid chromatography-tandem mass spectrometry (QTRAP) to quantify LMs and PUFAs in cerebrospinal fluid (CSF) and plasma from adult patients with pneumococcal meningitis and healthy controls. Patients had a median age of 62 years (IQR 50-69), and 37% experienced unfavorable outcomes (death, vegetative state, or severe disability). A marked shift in CSF PUFA composition was observed in patients, alongside the presence of 33 LMs that were undetectable in controls. The most abundant included 5-HETE, leukotrienes, thromboxane B_2, and prostaglandins. Levels of most oxylipins were substantially higher in CSF than in plasma, suggesting local production within the central nervous system. Among the 47 LMs identified in CSF, 23 were significantly elevated (≥2-fold) in patients with unfavorable outcomes. Notably, seven of these (5-HETE, 5-KETE, 5-HEPE, 5,15-diHETE, lipoxin A_4, 6-trans leukotriene B_4, and 6-trans-12-epi leukotriene B4) are direct products of 5-lipoxygenase, implicating this enzyme as an important metabolic driver of neuroinflammation. In vitro co-culture experiments with *Streptococcus pneumoniae* demonstrated that human macrophages produce LMs such as thromboxane B_2, and prostaglandins, but not 5-lipoxygenase-derived LMs. Post-mortem brain tissue from patients showed high expression of 5-lipoxygenase and 5-lipoxygenase-activating protein (FLAP) in infiltrating immune cells within the parenchyma and choroid plexus vasculature, suggesting these cells as the primary source of 5-lipoxygenase-derived LMs. In summary, this study provides a comprehensive characterization of LM metabolism in pneumococcal meningitis. We identify the 5-lipoxygenase/FLAP axis and macrophage-derived prostanoids as key contributors to intrathecal immunometabolism, linking specific lipid pathways to clinical outcome. These findings highlight LM metabolism as a potentially targetable driver of neuroinflammation in bacterial meningitis.

MULTIMARKER PROFILING OF INFLAMMATORY AND RESOLUTION MEDIATORS TO DIFFERENTIATE SEPTIC FROM CARDIOGENIC SHOCK

Marta Reina-Couto[1,2,3,4], Carolina Silva-Pereira[1,2], Patrícia Pereira-Terra[1,2], Sandra Martins[5], Luísa Teixeira-Santos[5], Dora Pinho[1,2], Miguel Luz Soares[7], Cláudia Camila Dias[8,9], João T. Guimarães[5,11,12], Roberto Roncon-Albuquerque[3,13], José-Artur Paiva[3,10], António Albino-Teixeira[1,2], Teresa Sousa[1,2]

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Background: Precision medicine envisions that identifying biological "phenotypes" will lead to personalized treatment (1). Cardiogenic (CS) and septic shock (SS) are both characterized by systemic inflammation, hemodynamic instability, and high mortality, but the specific roles of individual inflammatory components are not fully understood (2). Therefore, we evaluated a panel comprising cytokines, endothelial activation markers, inflammatory parameters, specialized pro-resolving mediators (SPMs) and their receptors to determine which one(s) better distinguish CS from SS. Methods: This ethics-approved study prospectively included patients with SS (n=20) and CS (n=25). Blood samples were collected at days 1–2, 3–4, and 5–8. Serum cytokines (TNF-alfa, IL-1β, IL-6, IL-10), endothelial activation markers (endocan, ICAM-1, VCAM-1, E-selectin), myeloperoxidase (MPO), and SPMs (LXA4; 15-epi-LXA4, RvD1, RvE1) were determined by multiplex immunoassays/ELISA. Chemerin1 and FPR2 mRNA expression in PBMCs was assessed by RT-qPCR. Differential blood cell counts and ratios (leucocytes, platelets, NLR and NMR) and C-reactive protein (CRP) were evaluated by automated methods alongside clinical/haemodynamic data and prognostic scores. Results: Principal component analysis (PCA)/partial least squares discriminant analysis (PLS-DA) models significantly differentiated ($p < 0.001$) CS and SS at admission ($p < 0.001$) and at days 3-4 ($p < 0.01$). E-selectin, NLR, NMR, MPO and FPR2 consistently discriminated SS from CS (VIP > 1) at both time points. TNF-alfa and CRP also were relevant discriminators at admission while total leucocytes and RvE1 became significant contributors at days 3-4. Univariate analyses showed at admission all discriminating variables except total leucocytes were significantly higher in SS. These differences persisted at days 3-4 except for TNF-alfa, CRP and NLR. Notably, at admission, E-selectin and RvE1 correlated significantly with hs-TnI only in SS. FPR2 positively correlated with pro-inflammatory markers in both groups, but with prognostic scores only in SS. Furthermore, in SS, lactate correlated positively with FPR2 and negatively with Chemerin1. Conclusions: A multimarker strategy including inflammatory and resolution biomarkers, significantly distinguishes SS from CS. Furthermore, FPR2 appears to drive a predominant pro-inflammatory response, and its correlation with lactate and prognostic scores points toward a potential therapeutic target in SS. Funded by FCT (PTDC/MEC-CAR/32188/2017) & COMPETE, Portugal 2020 (POCI-01-0145-FEDER-032188)

2nd Sponsored Session

Supported by The Lipidomics Research and Study Group (GERLI)

UNLOCKING OXYLIPIN AND PUFA PROFILING IN UNCONVENTIONAL BIOFLUIDS: CHALLENGES, PITFALLS, AND CLINICAL POTENTIAL

Denise Biagini[1], Silvia Ghimentia, Alessio Lenzia, Thierry Durand[2], Tommaso Lomonacoa, and Fabio Di Francescoa[3]

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The analysis of oxylipins and polyunsaturated fatty acids (PUFAs) in unconventional biological fluids offers unique insights into disease mechanisms and potential biomarkers, yet it presents distinct analytical challenges. This talk will provide a brief overview of the potentials and pitfalls associated with detecting and quantifying oxylipins and PUFAs in matrices beyond plasma and serum, including saliva, and dried blood spots (DBS). Emphasis will be placed on methodological considerations, such as sample stability, extraction efficiency, and sensitivity limitations, which can significantly impact data interpretation. A central focus will be on the MEPS-UHPLC-MS/MS platform developed in our laboratory, which enables efficient, high-throughput analysis of lipid mediators in small-volume samples. Several applications of this platform will be highlighted, demonstrating its versatility in profiling oxylipins and PUFAs across different biological matrices. Special attention will be given to the use of dried blood spots in Heart Failure, illustrating their feasibility for longitudinal monitoring, minimally invasive sampling, and integration into large-scale clinical studies. By combining the discussion of technological innovations with practical clinical applications, this talk aims to provide researchers with a clear understanding of when and how these approaches can be applied effectively, facilitating the translation of lipidomic measurements into meaningful clinical insights.

TARGETED SPATIAL METABOLOMICS FOR MAPPING LIPID MEDIATOR-RELATED INFLAMMATION IN THE LUNG

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Introduction Asthma is characterized by chronic inflammation in the lung that is mediated by a combination of immune and structural cells. These different cell populations synthesize lipid mediators (e.g., eicosanoids, octadecanoids), which impact asthma pathogenesis via controlled regulation of inflammatory processes. There is a need for methodology to determine the spatial heterogeneity of lipid mediator metabolism and the associated cell types in the lung. We present here a multi-modal method to perform combined targeted mass spectrometry imaging (MSI) and targeted spatial transcriptomics in lung tissue. Methods Lipid mediator distribution was mapped in human and mouse lung tissue using desorption electrospray ionization (DESI) coupled to targeted multiple-reaction-monitoring (MRM) (DESI-MRM) using a Xevo-TQ Absolute triple quadrupole. Single Cell Resolution IN Situ Hybridization On Tissues (SCRINSHOT) was subsequently conducted on the same cryosection to map RNA and profile cellular niches. An open-source workflow was implemented to process metabolite ion images from DESI-MRM data (quantMSImageR), annotate anatomical and cellular regions (CellProfiler) and map the annotations between ion and microscope images of different spatial resolutions and acquisition areas (napari based tools). The TissUUmaps open-source tool was used to combine targeted imaging modalities with histological staining, enabling integrated analyses of all imaging modalities. Results Spatial eicosanoid mapping revealed highly structured distributions in house dust mite (HDM)-challenged lungs. Products of cyclooxygenase (COX), including prostaglandin E2 (PGE_2) and 11-hydroxyeicosatrienoic acid (11-HETE), showed broad parenchymal distribution with airway enrichment, and together were significantly elevated in airways post-challenge ($p<10^{-13}$). Conversely, while the lipoxygenase (LOX) product 12-HETE also increased ($p<10^{-19}$), it displayed a distinct spatial pattern with weak PGE_2 correlation, indicating LOX-mediated formation in separate tissue microenvironments. This multi-modal workflow was able to sequentially co-register metabolites and mRNA distributions from the same cryosection, enabling visualization of metabolic and cellular responses to challenges. Results were integrated with histological staining to correlate metabolite and cellular patterns with physiological responses. Conclusions This targeted multi-modal integration enables routine visualization of the distribution of metabolites and associated cell type in the lung. These pathway-specific spatial distributions are indistinguishable by bulk analysis, demonstrating the power of spatial metabolomics to resolve compartmentalized eicosanoid biosynthesis during allergic airway inflammation.

TRIOXILINS ARE BACK: REDISCOVERING OVERLOOKED OXYLIPIN CHEMISTRY

Jean-Marie Galano, Ángel Sánchez-Illana, Guillaume Reversat, Huyanji Ji, Thierry Durand, Claire Vigor, Valérie Gros, Jiayin Dai, Yitao Pan

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Oxylipins derived from polyunsaturated fatty acids play central roles in human physiology, yet current knowledge remains largely focused on well-established families produced enzymatically and a limited number of autooxidation products. This apparent restriction is not solely biological but also analytical. Classical workflows, relying heavily on targeted approaches and the availability of reference standards, inherently bias detection toward known compounds. A growing body of studies has reported the formation of additional oxidation products that mimic enzymatically derived species, including highly oxygenated derivatives generated during lipid peroxidation. These compounds can represent a significant fraction of the reaction mixture, yet remain poorly characterized due to the lack of chromophores, analytical standards, and systematic MS MS annotation. High resolution mass spectrometry combined with molecular networking provides a framework to revisit this hidden chemical space by organizing MS MS data into structurally related molecular families and revealing diagnostic fragmentation patterns. Among these overlooked compounds, trioxilins emerge as a particularly compelling class. Trioxilins have been described in humans as downstream products of hepoxilin metabolism, with only a limited number of structures reported from linoleic, arachidonic, eicosapentaenoic, and docosahexaenoic acids. Their broader occurrence in human lipidomes remains largely unexplored. Here, we identify a characteristic MS MS fragmentation fingerprint shared by trioxilin A and trioxilin C types, enabling the detection of previously unannotated species. Using ex vivo oxidation of alpha linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids, we recover MS MS data corresponding to multiple unknown stereoisomers. Further analysis in human plasma and microalgae supports the existence of a broader family of trioxilin-related compounds. Thanks to MS2 data available they can now be systematically accessed using hybrid untargeted and targeted mass spectrometry strategies. Together, these results redefine trioxilins as a previously hidden but structurally coherent branch of oxylipin chemistry.

WHEN CONTAMINANTS MASQUERADE AS LIPIDS: BUILDING A CONTAMINANT LIBRARY TO PREVENT MISANNOTATIONS IN LIPIDOMICS

Carlos R. Canez, Bowen Yang and Liang Li

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An overlooked challenge in mass spectrometry-based lipidomics is the pervasive presence of contaminant signals originating from experimental workflows, such as labware contaminant leaching and solvent-derived contaminants. Unfortunately, these contaminants are frequently misannotated as endogenous compounds in untargeted lipidomic studies through putative high-resolution m/z matching. To address this issue, Lipid Contaminant Annotation Tool (Lipid CAT) was developed as an open-access resource systematically cataloguing contaminants commonly encountered in lipidomic workflows. The platform enables users to query experimental data, including tandem MS spectra, to distinguish contaminant-derived signals from analytes of interest, thereby improving the accuracy and reliability of untargeted lipidomic analyses. Borosilicate glassware has long been the standard material for lipidomic extractions due to its compatibility with organic solvents. Although some glassware manufacturers introduce higher contamination levels, established suppliers generally contribute less than 100 low-intensity contaminant signals. At present, plasticware has increasingly replaced glassware for lipid extraction and sample processing, among both experienced lipidomicists and newcomers. In contrast to glassware, polypropylene plasticware leaches substantial mass spectral contamination during Folch, MTBE, and BuMe biphasic extractions. Contamination profiles varied considerably among the four global polypropylene vendors evaluated. Notably, even the best-performing polypropylene tubes introduced 847 labware-derived contaminant m/z values, whereas less suitable tubes contributed thousands of contaminants per extraction. These polypropylene-derived contaminants caused extensive ion suppression of co-eluting endogenous lipids. Of particular concern, primary amide and fatty acid surfactants, indistinguishable from endogenous biological lipids, severely hindered quantification of their corresponding endogenous human lipid equivalents. Furthermore, contaminants may still be misidentified as endogenous lipids. In total, 1,476 plastic-derived contaminant m/z values were found within 5 ppm of SwissLipids or COMP_DB entries. Alarmingly, some of these contaminant signals have been erroneously reported in the literature as biologically significant lipid species. Lipid CAT represents the first open-access, lipidomics-tailored contamination library, systematically compiling thousands of contaminant m/z values and MS/MS spectra originating from plasticware, glassware, and analytical solvents. This resource enables rapid contaminant identification from m/z values and tandem MS data, empowering researchers to confidently distinguish contaminants from true lipid species and improve the reproducibility and overall data quality of untargeted lipidomics workflows.

COMPARATIVE EVALUATION OF MICROSAMPLING DEVICES FOR OXYLIPIN ANALYSIS IN NEWBORN BLOOD

Jose Luis Moreno-Casillas[1,2], Abel Albiach-Delgado[1,3], Mari Merce Cascant-Vilaplana[1], Alejandro Pinilla-Gonzalez[1,4], Laura Torrejon-Rodriguez[1,4], Laurence Balas[5], Thierry Durand[5], Guillermo Quintas[6], Julia Kuligowski[1,2], Maria Cernada[1,4]

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Study Background: Conventional clinical sampling methods typically rely on plasma or serum collection, requiring relatively large blood volumes, specialized handling procedures, and cold-chain storage. These limitations are particularly relevant in vulnerable populations and for the analysis of unstable, low-abundance lipid mediators such as oxylipins. In contrast, dried blood microsampling techniques require less than 100 µL of blood and offer advantages in terms of minimally invasive collection, sample stability, transport, and storage. This study aimed to develop and evaluate microsampling strategies suitable for routine clinical analysis as practical alternatives to conventional blood sampling methods. Outcomes: The performance of Pre-Cut Dried Blood Spots (PCDBS) and Volumetric Absorptive Microsampling (VAMS®) devices was investigated for oxylipin quantification, using liquid whole blood as the reference matrix. An LC-MS/MS method was developed and validated for the analysis. Both microsampling approaches employed 30 µL of fortified whole blood and samples were dried for 2 h prior to extraction. Improved analytical performance was obtained by preconditioning the devices with antioxidants before sample application and by preventing evaporation of internal standards during sample preparation. Stability during long-term storage was evaluated over three months, and both devices were applied to umbilical cord blood samples collected from 35 newborns. Comparable concentrations to those measured in liquid whole blood were observed for eight oxylipins using PCDBS and nine oxylipins using VAMS®, with mean concentrations ranging from 0.7 to 6.3 nM. Conclusions: This work represents the first application of VAMS® and PCDBS devices for oxylipin analysis in umbilical cord blood, demonstrating their suitability for quantification at nanomolar levels. The findings emphasize the importance of sample collection conditions, storage procedures, and internal standard handling in preserving lipidomic integrity. Sample stability proved to be highly dependent on storage temperature, making −20 °C preservation essential. Further studies should focus on protocol harmonization between laboratories and on improving LC-MS/MS sensitivity to broaden the range of detectable oxylipins.

Opening Plenary Lecture

Specialized Pro-resolving Mediator Pathways and Receptors In Orchestrating Resolution and Regeneration

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Resolution of the acute inflammatory response is a biosynthetically active process, governed by a superfamily of endogenous chemical mediators termed specialized proresolving mediators (SPMs), including resolvins (Rv), protectins and maresins. SPMs are resolution agonists, stimulating innate immune responses that promote resolution of inflammation, microbial clearance, tissue repair and regeneration. SPMs directly bind and activate specific cell surface G protein-coupled receptors (GPCRs) that initiate pro-resolving cellular and molecular mechanisms. SPM receptors were identified via unbiased screening of GPCR panels. We prepared radioactive-labeled ligands for each SPM to demonstrate specific binding to GPCR candidates, which gave affinities in the low nanomolar ranges, consistent with the effective concentrations for SPM bioactivities. The receptor-mediated pro-resolving signaling pathways and functions of SPMs were established in vitro and in vivo using several strategies, including receptor-deficient mice and gene silencing in inflammation-related pathologic conditions. These results confirmed that each identified SPM-receptor axis exhibits cell type- and organ-specific resolution signatures. Since SPMs are susceptible to rapid local enzymatic inactivation, metabolically stable SPM analogs were designed and synthesized. For example, a nouveau benzo-containing RvD2 mimetic activates the RvD2 receptor DRV2/GPR18, and that retains the bioactivities of RvD2 in stimulating phagocyte functions in vitro and in vivo, thus providing a manufacturable prototype for SPM mimetics. SPMs including RvD2 and thirteen-series resolvins (RvTs) exhibit potent actions in controlling bacterial infection via stimulating host phagocyte functions to clear microbes. In addition, these resolvins limit neutrophil extracellular traps (NETs) in human whole blood using microfluidic devices capturing NETs. The RvTs at nanomolar ranges decrease NETosis with isolated human neutrophils and in mouse infections, as well as enhance macrophage clearance of NETs. Together, SPMs, their receptors and pathways provide molecular basis for controlling excessive inflammation, stimulating endogenous resolution and tissue repair/regeneration, opening new opportunities to initiate resolution medicine.

Session 1

ROLE OF MACROPHAGE-DERIVED PROSTANOIDS IN HELMINTH INFECTION

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Macrophages are central regulators of protective immunity, inflammation resolution, and tissue repair during infection with multicellular helminth parasites. In previous work, we identified a helminth-derived glutamate dehydrogenase, heGDH, as an immunomodulatory protein that induces a regulatory eicosanoid shift and suppresses type 2 immune responses in allergic inflammation and helminth infection. Notably, helminth infection and heGDH treatment elicit a robust prostanoid response in macrophages and across multiple tissues, including the small intestine and bone marrow. In this talk, I will discuss how prostanoids shape host defense, macrophage reprogramming, and tissue repair during helminth infection Using hematopoietic- and macrophage-specific conditional deletion of cyclooxygenase-2 (COX-2) or microsomal prostaglandin E synthase-1 (mPGES-1), we uncovered distinct functional roles of prostanoid pathways in regulating macrophage states and downstream immune responses. In particular, our data reveal an unexpected role for PGE_2 in macrophage reprogramming via the bone marrow. I will present mechanistic insights into this regulatory circuit and discuss its implications for subsequent immune responses and tissue adaptation.

LIPID MEDIATOR, NOT LIPID MEDIATOR: REDEFINING BIOACTIVE LIPIDS BEYOND OXYLIPINS

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The term lipid mediator is commonly reserved for a relatively small group of oxidized fatty acid derivatives, including prostaglandins, leukotrienes, and related oxylipins. While these molecules play essential roles in inflammation and its resolution, this narrow definition overlooks a much broader landscape of lipids capable of actively shaping cellular physiology. In this presentation, I will challenge the traditional view of lipid mediators. Drawing on examples from sterol metabolism, and lipid-regulated transcriptional networks, I will highlight how numerous lipids influence cellular function, differentiation, and inflammatory responses despite rarely being classified as mediators. Particular focus will be placed on cholesterol biosynthesis intermediates and the emerging role of desmosterol as a signaling molecule. Beyond serving as the immediate precursor of cholesterol, desmosterol regulates key transcriptional pathways and modulates immune cell function, illustrating how metabolic intermediates can exert profound biological effects. Together, these findings argue for a broader and more inclusive definition of lipid mediators, one that extends beyond oxylipins and recognizes the diverse signaling functions embedded throughout lipid metabolism.

LOSS OF CD36 INDUCES DISEASE-ASSOCIATED OPCS IN THE BRAIN VIA PRO-INFLAMMATORY EICOSANOID SIGNALING IMPAIRING REMYELINATION

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Failure of remyelination is a major driver of axonal degeneration and disease progression in demyelinating brain disorders such as multiple sclerosis (MS). Growing evidence indicates that ineffective myelin repair stems from the inability of oligodendrocyte precursor cells (OPCs) to differentiate into mature, myelinating oligodendrocytes (OLNs), with metabolic dysfunction increasingly being recognized as an underlying cause. Here, we identified the fatty acid translocase cluster of differentiation (CD36), a member of the class B scavenger receptor family that regulates fatty acid homeostasis, as a key determinant of OPC maturation, remyelination, and neuroinflammation. We demonstrate that CD36 is markedly reduced in OLNs within chronic inactive MS lesions. Genetic ablation of CD36 impairs OPC differentiation in vitro and disrupts remyelination in both ex vivo cerebellar brain slice cultures and the in vivo cuprizone model. Guided by lipidomic and transcriptomic analyses, we further reveal that loss of CD36 leads to intracellular lipid accumulation and enhanced cyclooxygenase (COX-2)-mediated synthesis of pro-inflammatory eicosanoids. These changes reprogram OPCs towards an immune-reactive phenotype reminiscent of disease-associated OPCs, and prevent their differentiation into mature, myelinating OLNs. Importantly, treatment with 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC), a known CD36 ligand, enhances OPC differentiation in vitro and promotes remyelination ex vivo, highlighting a potential therapeutic avenue. Together, our findings establish CD36 as a metabolic gatekeeper in OPCs and uncover a lipid-mediated immune-metabolic checkpoint that governs remyelination in demyelinating disease.

CRACKING THE LIPID MEDIATOR CODE OF SEPTIC NEUTROPHILS

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Sepsis is a leading cause of U.S. mortality and hospital readmission, with annual costs exceeding $38 billion, yet no FDA-approved targeted therapies exist. It is driven by a dysregulated host response in which neutrophil programs become maladaptive, with dysregulated chemotaxis, impaired targeting of infection, and excessive tissue infiltration that fuels organ injury and immune paralysis. Lipid mediators (LMs) are rapid, potent regulators of neutrophil trafficking and immunometabolism: arachidonic acid-derived eicosanoids such as leukotriene B4 (LTB4) and prostaglandins drive mobilization and effector functions, while specialized pro-resolving mediators (SPMs) - including resolvins, protectins, and maresins - limit recruitment, promote apoptosis, and enhance macrophage-mediated efferocytosis. We hypothesized that LM signaling shapes neutrophil heterogeneity across sepsis progression by directing distinct behavioral and metabolic programs. To test this, we developed integrated imaging and computational pipelines to define the functional "behavioral" states of neutrophils at the single-cell level. Neutrophils were isolated from healthy donors and patient cohorts spanning ICU non-infectious controls, sepsis, and septic shock, and imaged by high-speed confocal microscopy; single cells were segmented and tracked, and >60 morpho-kinetic features were extracted to generate a 4D behavioral landscape. This approach resolved at least five distinct neutrophil behavioral states (B1-B5), spanning sessile/spherical, small migratory/amoeboid, small oblate, and large oscillatory phenotypes. LTB4 preferentially expanded the highly migratory amoeboid populations, consistent with LM-dependent control of neutrophil swarming, and this expansion was selectively counter-regulated by RvD4, which constrained these same migratory subsets without globally suppressing neutrophil activity. Strikingly, RvD4 reactivated phagocytosis in septic neutrophils, restoring antimicrobial function that is otherwise lost as disease severity progresses. Sepsis and septic shock neutrophils displayed distinct, severity-specific behavioral signatures consistent with progressive immune paralysis, with septic shock cells showing the most profound shift toward dysfunctional states. Targeted LC-MS/MS revealed concurrent lipidome remodeling, and impaired mitochondrial fatty-acid utilization and increased neutral lipid storage. Behavioral heterogeneity co-occurred with this metabolic remodeling, supporting linked regulation of trafficking phenotypes and immunometabolic state. Together, these findings show that LM signaling - via LTB4 and RvD4 acting on specific neutrophil subpopulations, couples behavioral and metabolic programs, providing a translational framework to stratify sepsis heterogeneity and advance resolution-directed biomarkers and therapies.

EPA+DHA AND THEIR MONOHYDROXY-OXYLIPIN METABOLITES INCREASE MITOCHONDRIAL RESPIRATION CAPACITY IN M0 BUT NOT M1 MACROPHAGES

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*Helena L Fisk is first and senior corresponding author. This is one of her first PI projects independently securing funding during a personal fellowship award period where all work (lipidomics under the support and guidance of Dr Tyrrell and Prof O'Donnell with both playing key roles in data quality control/validation) was conducted by Fisk.

Introduction: Obesity is characterised by chronic low-grade inflammation, which contributes to insulin resistance and cardiovascular disease. Adipose tissue macrophages play a role in this through the secretion of mediators including oxylipins that influence adipocyte function. Previous work by Fisk demonstrated reduced levels of DHA-derived oxylipins, alongside accumulation of pro-inflammatory (M1-like) macrophages in subcutaneous adipose tissue in human obesity, which were not modified by dietary omega-3 fatty acid (EPA+DHA) supplementation. Direct oxylipin supplementation may offer an alternative strategy to restore oxylipin profiles and reduce associated inflammation. This project aims to examine the effects of a mixture of the oxylipins 14-HDoHE (0.03 μm) + 17-HDoHE (0.11μm) + 18-HEPE (0.02μm) (OXLP), alone or combined with EPA+DHA (30+20μm), on homeostatic (M0-like) and M1-like macrophages and in co-cultures with primary murine adipocytes. Here, findings from isolated macrophages are presented. Methods: M0- and M1-like macrophages were cultured with EPA+DHA, OXLP, or both for 48-hours. Cell fatty acid composition was analysed by gas chromatography; supernatant oxylipins by UPLC-MS/MS; and mitochondrial respiration by Seahorse respirometry. Results: EPA+DHA and OXLP increased basal and maximal respiration, ATP production and proton leak ($P<0.026$) in M0-like macrophages only. Inflammatory polarisation of macrophages to M1-like phenotype resulted in a trend for reduced maximal respiration ($P=0.055$) and proton leak ($P=0.068$) by ~50% each. EPA+DHA increased total cell EPA+DHA content ($P<0.002$), and supernatant levels of several oxylipins (HEPEs, HDoHEs, EpDPAs, 5,6,15-triHETE, PGD_3, and PGE_3) for both macrophage phenotypes ($P<0.05$). OXLP increased 17-HDoHE only. Media controls highlighted non-enzymatic oxidation of EPA+DHA which was further elevated in cell experiments. Using the combination of EPA+DHA+OXLP resulted in a blunted increase in non-enzymatic oxidation of EPA+DHA. Combined EPA+DHA+OXLP did not produce additive effects beyond EPA+DHA alone for any outcome. Conclusion: Treatment of macrophages with EPA+DHA or OXLP in culture modifies homeostatic macrophage respiration. Treatment with EPA+DHA results in non-enzymatic oxidation of EPA, DPA, and DHA to -derived oxylipins. These findings suggest EPA+DHA and OXLP have potential to alter mitochondrial respiration of selected macrophage populations which may have downstream effects for macrophage-adipocyte communication and obesity associated inflammation. Adipocyte-macrophage co-culture experiments are underway to explore this further.

Session 2

STRUCTURES OF INHIBITOR-BOUND 15-PGDH: INSIGHTS INTO MOLECULAR MECHANISMS AND REGENERATIVE THERAPEUTIC OPPORTUNITIES

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Prostaglandin E_2 (PGE_2) is a lipid signaling molecule that plays a key role in inflammation and tissue regeneration. PGE_2 initially acts as a pro-inflammatory mediator but transitions to support the resolution phase by promoting the biosynthesis of specialized pro-resolving mediators (SPM) like lipoxins, which aid in tissue repair. The enzyme 15-PGDH negatively regulates tissue stem cell differentiation by oxidizing and degrading PGE_2, thus modulating its availability during tissue repair. Inhibiting 15-PGDH markedly increases PGE_2 concentrations to accelerate tissue repair across multiple organs and promotes increased muscle mass in aging models. We have used cryo-electron microscopy to solve the solution structure of native 15-PGDH and its complexes with two distinct chemical inhibitors, achieving ~2 angstroms resolution. Our structural analysis identifies key residues, including F185 and Y217, that regulate a dynamic lid domain crucial for the enzyme's function. These inhibitors exploit a unique hinge mechanism regulated by these residues to capture the lid of 15-PGDH in a closed conformation and thereby explaining the tight, sub-nanomolar binding affinities of the chemical inhibitors. These findings provide a foundation for the fundamental mechanisms involving 15-PGDH-mediated prostaglandin inactivation and for developing 15-PGDH targeted drugs as therapeutics in regenerative medicine and anti-aging strategies, enhancing PGE_2's beneficial effects by preventing its degradation and supporting tissue repair. Additionally, we have implemented MM-PBSA to further define binding contributions of individual protein residues surrounding the ligand binding pocket, guiding the development of new inhibitors with different chemical scaffolds.

DECODING RESOLUTION BIOLOGY: NEW INSIGHTS INTO MOLECULAR DYSREGULATION DRIVING DISEASE PROGRESSION

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Chronic inflammatory diseases are increasingly recognised not simply as disorders of excessive immune activation, but as conditions driven by dysregulation of endogenous resolution programmes that normally restore tissue homeostasis following injury or infection. Emerging evidence indicates that specialised pro-resolving mediators (SPMs) act as critical regulators of immune cell programming, tissue protection, and inflammatory adaptation, and that disruption of these pathways contributes directly to disease progression. Recent studies have uncovered previously unrecognised tissue-specific circuits through which SPM pathways coordinate inflammatory homeostasis across distinct organ systems. In the intestine, resident macrophage-derived lipid mediators regulate epithelial barrier integrity and constrain pathogenic cytokine-driven inflammatory responses, thereby limiting susceptibility to chronic intestinal inflammation. In parallel, studies within the bone marrow have identified erythroblasts as key contributors to local SPM biosynthesis that shape granulopoiesis and imprint neutrophil functional identity through niche-dependent mechanisms. Disruption of these pathways results in maladaptive immune cell phenotypes associated with impaired host defence, exaggerated inflammatory responses, and increased tissue damage. Together, these findings support a broader paradigm in which resolution biology functions not merely as a passive termination programme, but as an active tissue-organising network that controls immune cell development, inflammatory thresholds, and disease susceptibility. Understanding how these endogenous pathways become dysregulated in chronic disease may provide important opportunities for the development of next-generation therapeutics aimed at restoring immune homeostasis and tissue protection rather than broadly suppressing inflammation.

FUNCTIONAL LIPID MEDIATOR SCREENING IDENTIFIES PROSTAGLANDIN D2 AS SUPPRESSOR OF MPGES-1 EXPRESSION VIA THE KEAP1-NRF2 AXIS IN MACROPHAGES

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Background: Prostaglandin E_2 (PGE_2) is a central regulator of inflammatory responses, contributing to both the initiation and the resolution phase. Its biosynthesis critically depends on coordinated induction of cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1). Local crosstalk within lipid mediator networks can augment or suppress these enzymes and thereby shape the magnitude and duration of PGE_2 production at sites of inflammation. Methods: Lipid mediator networks in primary human monocyte-derived macrophages were profiled by targeted and untargeted metabololipidomics (UHPLC-MS/MS) and correlated with expression kinetics of prostanoids biosynthetic enzymes at mRNA and protein levels. A panel of lipid mediators including specialized pro-resolving mediators (SPM) and their precursors, leukotrienes, and prostanoids (or stable analogues thereof), was screened for modulatory effects on PGE_2 formation. Pharmacological approaches were employed to distinguish receptor-mediated versus electrophile-driven mechanisms. Results: While canonical SPM and other lipoxygenase-derived LM were inactive in this reductionist system, PGD_2 significantly interfered with PGE_2 production in M1-like macrophages. PGD_2 and its downstream metabolites selectively suppressed mPGES-1 expression with comparatively minor effects on COX-2. Notably, induction of COX-2 and mPGES-1 was driven by LPS during classical macrophage polarization; however, their kinetics were markedly different, with rapid COX-2 upregulation (within hours) versus delayed mPGS-1 expression (t_{max} > 24 h), defining a temporally distinct "late PGE_2 axis." Mechanistically, PGD_2 acted independently of DP1/DP2 receptor signaling and required metabolic conversion into electrophilic cyclopentenone PGs of varying potency and cytotoxicity. Although the PPARγ-ligand 15-deoxy-Δ12,14-PGJ_2 contributed to the response, suppression of mPGES-1 was largely independent of PPARγ. Instead, direct modulation of Keap1 and subsequent activation of Nrf2 appeared as the dominant mechanism, leading to antioxidant reprogramming and selective repression of mPGES-1/PGE_2 formation. This regulatory axis of PGD_2 blocking LPS-induced mPGES-1 expression was conserved across leukocyte populations, including neutrophils. Conclusions: PGD_2 and its metabolites act as intrinsic negative modulators of mPGES-1-derived PGE_2 biosynthesis potentially via electrophile-driven activation of the Keap1-Nrf2 pathway. These findings highlight SPM-independent mechanisms of inflammation control and position PGD_2 metabolites as endogenous drivers of macrophage reprogramming. Targeting the temporally distinct mPGES-1 arm of prostanoids biosynthesis - while preserving anti-inflammatory signaling - may complement emerging resolution-based therapeutic strategies.

NOVEL LysoPS - GPCR SIGNALLING IN B CELL RESPONSES

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Extracellular lipid mediators regulate immune cells through binding to G-protein coupled receptors (GPCRs). GPCRs exert their various effects by the activation of downstream signalling molecules such as trimeric G alpha proteins and are prominent drug targets. Recently, a new class of lipid mediators based on lysophosphatidylserine (LysoPS) has been discovered and associated with autoimmunity. However, which specific receptors regulate B cell function or pathology remains elusive. We have recently identified three GPCRs binding the LysoPS as inhibitors of B cells in genome-wide CRISPR screens. Given the growing interest in lipid mediators as pharmacological targets and the data we have gathered, we aim to explore how LysoPS shapes B cell responses to antigens. Our novel data on GPR174 and P2RY10 hints at a more diversified role of LysoPS signalling on B cells than previously anticipated. Our work demonstrates how this set of GPCRs regulates B cell activation, survival, proliferation and plasma cell differentiation, using both in vitro as well as in vivo models.

YOUNG INVESTIGATOR SESSION

ORAL ABSORPTION, BIOAVAILABILITY, AND UPTAKE ROUTES OF OMEGA-3 POLYUNSATURATED FATTY ACID DERIVED OXYLIPINS IN RATS

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Background: Oxylipins derived from omega-3 polyunsaturated fatty acids (n-3 PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are critical mediators of the resolution of inflammation. In contrast to the well-established oral absorption of EPA and DHA, the bioavailability of their oxylipin derivatives remains unstudied. This research used stable isotope labelling and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to investigate the oral absorption of n-3 PUFA derived oxylipins in rats. Objectives: This study aimed to: (1) determine whether oxylipins are absorbed after oral intake, (2) calculate their bioavailability, and (3) determine the mechanism by which they are absorbed. Methods: Stable isotope 13C-labelled n-3 PUFA and deuterium labelled-oxylipins were administered through oral, intravenous (IV), and intraduodenal routes in male Sprague-Dawley rats. Co-administration of 13C-labelled PUFA and deuterated oxylipins differentiated between endogenously synthesized oxylipins (unlabelled), synthesis of oxylipins from absorbed n-3 PUFA precursors(13C-labelled), and direct oxylipin absorption (deuterium-labelled). IV administration allowed for the calculation of clearance kinetics and absolute bioavailability. Intraduodenal administration with mesenteric lymph duct cannulation enabled the direct assessment of lymph transport as a potential mechanism of absorption. Blood and lymph samples were collected and analyzed by LC-MS/MS. Results: Orally administered oxylipins were rapidly absorbed into the unesterified plasma pool, reaching peak concentrations within 15 min to 1 h, with a bioavailability of 3-6%. In contrast EPA and DHA had much higher bioavailability in the unesterified pool of 66% and 42% respectively. No endogenous synthesis of 13C-labelled oxylipins from absorbed n-3 PUFA precursors was detected. IV administration revealed rapid plasma clearance of both n-3 PUFA and oxylipins (half-life ~30 s). Trihydroxy oxylipins exhibited lower recovery in mesenteric lymph compared to EPA and monohydroxy oxylipins. Furthermore, lymph cannulation reduced plasma appearance of EPA and monohydroxy but not trihydroxy oxylipins, indicating structure-dependent changes in absorption via lymphatic or portal routes. Significance: These findings demonstrate that n-3 PUFA-derived oxylipins are absorbed following oral intake in rats. Defining their bioavailability, rapid clearance, and distinct absorption mechanisms provides a foundation for the oral delivery of these novel therapeutics.

INHIBITION OF THE SOLUBLE EPOXIDE HYDROLASE IMPEDES IMMUNOSUPPRESSIVE RESPONSES IN MICE WITH CCL4-INDUCED CIRRHOSIS

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Background: Patients with acutely decompensated (AD) cirrhosis present persistent immunosuppression rendering them at increased risk of recurrent bacterial infections and developing acute-on-chronic liver failure (ACLF). Here, we investigated the profile of immunomodulatory lipid mediators in patients with AD cirrhosis, and we explored the effect of inhibition of the soluble epoxide hydrolase in a model of CCl4-induced cirrhosis in mice. Methods: Targeted lipidomics of 101 well-annotated lipid mediators was performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in 308 samples of plasma from 93 AD patients with and without ACLF at hospital admission and during 90-day follow up. For comparison, 31 healthy donors were also included. Immune responses were assessed in vitro in mononuclear leukocytes. In vivo studies were performed in peritoneal and liver macrophages from mice with CCl4-induced cirrhosis to assess the immunosuppressive profile by flow cytometry immunophenotyping after soluble epoxide hydrolase inhibition treatment. Results: LC-MS/MS analysis identified the linoleic acid-derived 9,10-dihydroxy-12-octadecenoic acid (9,10-DiHOME), also known as leukotoxin, as the only lipid mediator specifically elevated in ACLF patients in comparison to those without. Moreover, follow-up analysis revealed that 9,10-DiHOME levels followed the severity course of the disease and were significantly higher at the time patients developed an active infection and at ACLF presentation. Consistent with these findings, leukocytes from AD patients showed increased expression of soluble epoxide hydrolase (sEH), the enzyme responsible for 9,10-DiHOME biosynthesis. In in vitro assays, 9,10-DiHOME induced the expression of the immunosuppressive marker MerTK, distorted required mechanisms for the adequate host defense response against pathogens including the ability to produce cytokines in response to LPS and impaired mitochondrial dynamics and autophagic responses. In in vivo studies, inhibition of sEH in mice with CCl4-induced cirrhosis impeded immunosuppressive responses in peritoneal macrophages and significantly attenuated MerTK expression in liver macrophages. Conclusion: Taken together, these findings indicate that increased circulating levels of the leukotoxin 9,10-DiHOME weakens immune-cell defensive responses, thus enhancing the susceptibility of AD patients to bacterial infection and precipitating ACLF. These data also position sEH as an actionable drug target in this condition.

PLAYER N°3 HAS ENTERED THE GAME: DPA AS A MODULATOR OF PULMONARY VASCULAR TONE

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Introduction: Pulmonary hypertension (PH) is a severe disease characterized by progressive pulmonary vascular remodeling, increased pulmonary arterial pressure, and right ventricular failure. Current therapies mainly target vasoconstriction but remain insufficient to control disease progression. Increasing evidence suggests that omega-3 fatty-acids and their metabolites, specialized pro-resolving mediators (SPMs), modulate vascular tone [2, 3], prostanoid signaling, and inflammatory pathways relevant to PH [4]. However, whereas EPA and DHA dominate the omega-3 literature, docosapentaenoic acid (DPA), the intermediate omega-3 fatty-acid, represents only 2.8% of the omega-3 literature (1,018 publications vs 14,484 for EPA and 20,237 for DHA, May 2026). We hypothesized that DPA and its derived mediators modulate pulmonary vascular tone and smooth muscle cell migration in PH. Methods: Human pulmonary arteries (HPA) from control and Group 3 PH patients were collected at Bichat Hospital. Endogenous lipid mediator production was assessed by LC–MS/MS lipidomics following short ex vivo DPA stimulation (10 µM, n=3 control donors). Vascular reactivity to EPA, DHA, and DPA, alone or combined with PGE_2, was evaluated using an organ-bath system (n=5–7). Adrenergic vasoconstriction was assessed using norepinephrine. EP3 and TP receptor expression were analyzed by western blot (n=5–7). Migration assays were performed on human pulmonary arterial smooth muscle cells (hPASMCs) from Group 3 PH patients (n=1–4) using omega-3 fatty-acid, DPA-derived SPMs, and iloprost, a prostacyclin analogue. Results: DPA stimulation induced de novo production of T-series resolvins (RvTs), undetectable at baseline. Functionally, DPA induced vasorelaxation in control arteries, an effect attenuated in PH arteries. Unlike EPA or DHA, DPA also reduced norepinephrine-induced vasoconstriction. DPA more potently attenuated PGE_2-induced vasoconstriction compared with EPA and DHA, reducing PGE_2 Emax by ~66%, in parallel with reduced EP3 receptor expression. In hPASMCs, DPA alone did not affect migration, whereas co-incubation with iloprost potentiated its anti-migratory effect. Exploratory experiments identified RvT3 (1 nM) as a potential mediator, whereas RvT1, RvT4, and RvD2n-3DPA showed no detectable effect. Conclusion: These findings identify DPA as a previously overlooked modulator of pulmonary vascular function in PH. DPA-derived pathways, including RvTs production and modulation of prostanoid- and adrenergic-dependent responses, emerge as novel mechanisms in pulmonary vascular biology.

PLATELET-ACTIVATING FACTOR IN MICROBIAL KERATITIS: INSIGHTS FROM A ZEBRAFISH LARVAE CORNEAL INJURY MODEL

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Microbial keratitis (MK), an infection of the cornea, is a major global cause of visual impairment and blindness, affecting an estimated 1.5–2 million individuals annually. It is also the second most common cause of unilateral blindness after cataracts. Disease severity is mediated not only by microbial infection but also by a dysregulated inflammatory response that drives corneal thinning and scarring, culminating in permanent visual loss. Despite this, effective immunomodulatory therapies remain limited. Platelet-activating factor (PAF) is a potent phospholipid mediator implicated in inflammatory processes; however, its role and biosynthesis in MK pathogenesis remain poorly defined. We hypothesised that PAF amplifies the immune response in MK by promoting leukocyte recruitment. To investigate this, we utilised a recently established zebrafish larval corneal injury and infection model (1), enabling real-time in vivo visualisation of immune cell dynamics at high spatiotemporal resolution. Experiments were performed in transgenic zebrafish larvae, Tg(mpx:GFP;mpeg1:mCherry), in which neutrophils and macrophages are fluorescently labelled. This system was interrogated using complementary pharmacological and genetic approaches. Exogenous PAF significantly increased neutrophil and macrophage recruitment to the injured cornea. This response was abolished by the selective PAF receptor antagonist WEB 2086 ($P = 0.0002$), indicating receptor-dependent signalling. Consistently, CRISPR–Cas9-mediated knockdown of the PAF receptor reduced immune cell recruitment, and this phenotype was not rescued by exogenous PAF ($P = 0.0153$), confirming that the pro-inflammatory effects of PAF are mediated via its canonical receptor. We next examined the role of endogenous PAF biosynthesis. Knockdown of lysophosphatidylcholine acyltransferase (LPCAT) isoforms revealed a selective role for LPCAT2 ($P < 0.001$), but not LPCAT1 ($P = 0.2604$), in regulating leukocyte recruitment. The reduction in recruitment following LPCAT2 knockdown was rescued by exogenous PAF ($P < 0.0001$). In line with these findings, pharmacological inhibition of LPCAT2 using TSI-01 resulted in a dose-dependent decrease in immune cell infiltration at the injury site ($P < 0.0001$). Collectively, this study identifies PAF as a key regulator of innate immune cell recruitment in MK and demonstrates that LPCAT2-dependent PAF biosynthesis is a critical upstream mechanism. These findings highlight the PAF signalling axis as a promising therapeutic target for modulating inflammation and limiting tissue damage in microbial keratitis.

1 Cheng, K. K. W. et al. Real-time characterisation of microbe-induced inflammation using a novel zebrafish larval corneal injury and infection model. Communications Biology (2026). https://doi.org:10.1038/s42003-026-09985-1

INHIBITION OF ENDOCANNABINOID DEGRADATION ACCELERATES POST-OPERATIVE PAIN RESOLUTION

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Postoperative pain is an expected immediate consequence of surgery. However, in 10-50% of patients, pain persists and progresses to chronic postoperative pain. Current treatments, including NSAIDs and opioids, mainly provide symptomatic relief and fail to promote pain resolution. The endocannabinoid system controls various processes at the interface between the immune and nervous systems. For instance, modulation of endocannabinoid levels through inhibition of their main hydrolytic enzymes, monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), has been shown to reduce pain in several contexts. However, their contribution to postoperative pain recovery remains poorly understood. The aim of this study was to determine whether targeting endocannabinoid hydrolysis could promote recovery from postoperative pain beyond conventional analgesic effects. Male mice underwent hind-paw incision (HPI) to model postoperative pain. Daily administration of treatments (the MAGL inhibitor JZL184, the FAAH inhibitor PF04457845, the dual MAGL/FAAH inhibitor JZL195 or vehicle) was initiated 3 days after surgery. Mechanical nociception was assessed longitudinally to evaluate pain recovery. In parallel, a transcriptomic analysis of dorsal root ganglia and sciatic nerves was performed. To investigate the mechanisms underlying pain recovery, cannabinoid receptor involvement was assessed using a selective receptor antagonist in combination with MAGL inhibition. Inhibition of MAGL significantly accelerated recovery from mechanical postoperative nociception. Mice treated with JZL184 and JZL195 returned to baseline mechanical sensitivity approximatively five-days before vehicle-treated animals. In contrast, FAAH inhibition produced only a transient reduction of postoperative nociception, limited to the first day of treatment, with no sustained effect on pain recovery. The accelerated resolution induced by MAGL inhibition was associated with altered expression of genes involved in inflammatory and nociceptive pathways. Finally, we show that the effects of MAGL inhibition were mediated by activation of the CB2 receptor as they were fully blocked by a selective CB2 antagonist. These data suggest that MAGL inhibition and increasing 2-AG levels could promote the resolution of postoperative pain. This discovery could lead to a new, more comprehensive approach to postoperative pain management that goes beyond merely reducing symptoms.

RESOLUTION OF INFLAMMATION AND SPECIALIZED PRO-RESOLVING LIPID SIGNALING ARE ALTERED IN THE Scnn1b TRANSGENIC MICE MODEL AND CAN BE CORRECTED BY RVD1 TREATMENT

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Introduction Cystic fibrosis (CF) is characterized by an altered mucociliary clearance driving inflammation and airway tissue degradation. In addition, the biosynthesis of several Specialized Proresolving Mediators (SPM), including lipoxins (LX), resolvins (Rv) and maresins (MaR) is decreased in CF (Karp, 2004, Ringholz 2014, Shum, 2022). This alteration may contribute to persistent inflammation. In this study, we explored the resolution biology including SPM biosynthesis and function, SPM receptor expression, immune cell infiltration, mucus accumulation in a murine model of CF. Methods We use Scnn1b transgenic mice (Tg) modelling the mucus dehydration and inflammation described in the airway of CF patients (Mall, 2004). In broncho-alveolar lavage (BAL), we evaluate immune cell infiltration by flow cytometry and histology, mucus secretion by PAS-AB staining. In lung, we quantified SPM content by LC-MS/MS (Ambiotis) and their receptor expression by RT-qPCR. Results Tg mice displayed alveolar damage, mucus accumulation and mucin over-expression in the mung. Tg BAL analysis showed neutrophilic infiltration and decreased levels of macrophages involved in repair processes (e.i. Ly6G pos). Furthermore, the transcription of the SPM receptors, ChemR23, GPR18, GPR37 and overall SPM biosynthesis were disrupted in Tg lungs, characterized by a significant decrease in LXA4 and MAR2 which were consistent with increased 5-LOX and decreased 12-LOX activities. Moreover, preliminary analyses showed that SPMs levels tend to further decrease in aging mice (12 months) and in female. Finally, treatment of Tg mice with RvD1 during two weeks (intraperitoneally, 100ng, 3 times a week, Ferri 2023) reduced mucus accumulation and muc5b and muc5ac transcription in the lung. Neutrophil levels decreased, pro-repair macrophages populations increased and SPM receptors transcription levels tend be corrected, reflecting a less severe inflammation in Tg lung. Conclusion Our results highlight alterations in the resolution biology including pro-repair immune cell types, SPM biosynthesis and function in Scnn1b Tg mice that can be prevented by RvD1 treatment.

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INTERFERON-β INDUCES LIPID MEDIATOR CLASS SWITCHING TO DRIVE RESOLUTION OF BACTERIAL AIRWAY INFLAMMATION

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Neutrophil dysfunction underlies acute respiratory distress syndrome (ARDS). We previously showed that bacterial and mitochondrial DNA (CpG DNA) can evoke neutrophil dysfunction, impair bacterial clearance and prolong lung injury. Interferon-β (IFN-β) has been implicated in host defense against bacteria; however, little is known about its role in the regulation of lipid mediator pathways during ARDS. We investigated the impact of IFN-β on synthesis of LTB_4 and selected specialized pro-resolving lipid mediators (SPMs). Using a murine model of self-limited *E. coli*-evoked airway inflammation, we demonstrate IFN-β regulation of the temporal production of lipid mediators. CpG DNA or neutralizing endogenous IFN-β markedly increased bronchoalveolar lavage fluid levels of LTB_4 and reduced 15-epi-LXA_4 and RvD1. Conversely, treatment with IFN-β increased lavage fluid levels of 15-epi-LXA_4 and RvD1 and reduced LTB_4. Furthermore, selective blockade of ALX/FPR2 (which binds both 15-epi-LXA_4 and RvD1) with WRW4 partially blocked IFN-β-mediated resolution and impaired bacterial clearance. IFN-β promoted efferocytosis and reduced neutrophil persistence in the inflamed lung. In contrast, neutralizing endogenous IFN-β delayed clearance of apoptotic neutrophils, and prolonged inflammation. Importantly, treatment of mice with 15-epi-LXA_4 or 17-epi-RvD1 at the peak of inflammation rescued defective resolution responses. Mechanistically, culture of human neutrophils with CpG DNA generated survival cues, impaired phagocytosis and enhanced LTB_4 production through stimulating translocation of 5-lipoxygenase (5-LOX) to the nucleus. IFN-β countered the survival cues from CpG DNA but did not restore phagocytosis. IFN-β partially prevented 5-LOX translocation to the nucleus, resulting in reduced LTB_4 generation and slight increases in 15-epi-LXA_4 and RvD1 production. Culture of neutrophils with exogeneous 15-epi-LXA_4 or 17-epi-RvD1restored impaired phagocytosis by CpG DNA and enhanced bacterial killing. These findings identify IFN-β as an inducer of lipid mediator class switching, orchestrating resolution of inflammation through SPM and suggest a therapeutic potential of harnessing the IFN-β-SPM axis for enhancing the resolution of neutrophil-driven ARDS. (Grant support: CIHR MOP-97742 and MOP-102619).

UNTARGETED LIPIDOMICS IN VISCERAL ADIPOSE TISSUE REVEALS LIPID ALTERATIONS IN INDIVIDUALS WITH OBESITY AND VARYING GLYCEMIA

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Background: Adipose tissue (AT) is heterogeneous, with visceral AT (VAT) and subcutaneous AT (SAT) differing in insulin sensitivity and lipid handling. Accumulation of fatty acids (FA) during obesity and type 2 diabetes (T2D) may impair AT function, yet the contribution of depot-specific lipid composition to metabolic dysfunction remains unclear, warranting further investigation. Objective: To examine how the FA profile, lipid fractions and species differ in AT depots from individuals with obesity and hyperglycemia. Methods and Results: Thirty VAT and SAT samples from bariatric surgery donors with a BMI ≥ 30 kg/m2 and either normoglycemia or hyperglycemia were assessed. For the total FA profile, the concentration (µmol/g) of arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) was higher in the SAT depot compared to VAT; ARA and DHA concentrations were higher in hyperglycemia compared to normoglycemia group. Further analysis in six different lipid fractions including triacylglycerol (TG), diacylglycerol (DG), monoacylglycerol, free fatty acids, phospholipid (PC) and cholesterol esters exhibited similar increases in ARA, EPA, and DHA in the SAT compared to the VAT depot and in the hyperglycemia group. Given the role of ARA, EPA and DHA as substrates for cyclooxygenases, lipoxygenases and cytochrome P450 pathways, the altered PUFA distribution may influence downstream lipid mediator production and species. Untargeted lipidomics was conducted where extracted lipids were analyzed in both negative and positive ionizations modes using the LC-MS/MS. LipidMatch suite 5.4 was used for lipid identification, and the peak areas were normalized to class-specific deuterated internal standards (SPLASH LipidoMix). Preliminary data based on heatmaps generated in MetaboAnalyst suggests hyperglycemia group having more abundant lipid species compared to normoglycemia in the VAT depot. Specific lipid species include a few DHA containing TGs such as TG(18:1_22:6_22:6), TG(18:0_18:0_22:6), a palmitate DG(16:0_16:0) and ARA containing OxPC(20:4_18:1). Further analysis in the SAT depot are required to understand depot-specific and glycemia based lipid compositional changes. Significance: These findings highlight that VAT and SAT depots store lipids differently depending on the glycemic status in obesity. Future objectives include assessing oxylipins in these samples and using in vitro adipocyte models to understand their contribution to AT function.

CHEMOPROTEOMIC MAPPING OF N-ACYL TAURINE SIGNALLING IN PRIMARY HEPATOCYTES

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N-acyl taurines (NATs) are an emerging class of lipids that regulate metabolic homeostasis. NATs consist of a long-chain fatty acid conjugated to taurine and are synthesised in the liver and kidney. Human cohort studies have shown circulating NATs increase with metabolic stress, while preclinical models indicated that elevated NATs reduce lipid accumulation, inflammation and insulin resistance. However, the molecular mechanisms and signalling pathways underlying these effects remain poorly defined, limiting their translational potential. This study employed a chemoproteomic approach to identify NAT-protein interactions in primary hepatocytes isolated from female C57BL/6 mice. Cells were treated with either a photoaffinity-labelled NAT or the corresponding free fatty acid, enabling crosslinking and enrichment of interacting proteins followed by proteomic analysis. This approach allowed selective identification of NAT-associated proteins, as well as comparisons between the interaction profiles of the NAT and fatty acid. Results showed NATs specifically interacted with pyruvate kinase, PKM, a key enzyme in glycolysis. In addition, mitochondrial proteins were enriched in NAT-treated cells compared to the free fatty acid control. Follow-up studies using lipopolysaccharide to induce metabolic stress demonstrated a ~2-fold increase in NAT-protein interactions, suggesting that NAT signalling may be remodelled in response to inflammatory stimuli. Together, these findings establish a platform for identifying NAT targets in physiologically relevant systems and provide initial evidence for distinct NAT signalling networks.

MARESIN 1 REMODELS HEPATIC TRANSCRIPTOME, ATTENUATING INFLAMMATION AND MITOCHONDRIAL DYSFUNCTION IN AGED FEMALE MICE WITH OBESITY AND MASLD

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is a major global health challenge, particularly affecting aging populations and individuals with obesity, with women facing increased risk after menopause. The high prevalence and serious consequences of MASLD, including progression to steatohepatitis (MASH), cirrhosis, liver failure, and increased cardiovascular risk, underscore the critical need for effective therapeutic strategies. Specialized pro-resolving mediators, such as Maresin 1 (MaR1), have emerged as promising candidates due to their demonstrated anti-inflammatory and pro-resolving capabilities. This study aimed to elucidate the therapeutic potential and underlying mechanisms of MaR1 in ameliorating hepatic inflammation and mitochondrial dysfunction in a model of aged female mice with obesity and MASLD. Two-month-old C57BL/6J female mice were fed ad libitum with a high fat diet (HFD, 45%) for 15 months to induce obesity and MASLD. Then, HFD-fed mice were daily treated (i.p.) with MaR1 (15 µg/kg) or vehicle for 20 consecutive days. Body weight, body composition, GTT, ITT and serum biochemical assays were assayed. Liver samples were obtained, weighed, and histological analyses were carried out. Transcriptomic profile of liver samples was analysed by RNAseq. Oxygen consumption rate was evaluated in isolated liver mitochondria by Seahorse. MaR1 administration significantly improved glucose tolerance without significantly affecting body weight or fat mass. MaR1 moderately reduced hepatic triglyceride content and TBARS levels. Transcriptomic analysis revealed that MaR1 orchestrated a robust remodelling of the hepatic gene expression profile. GO enrichment analysis revealed that liver of MaR1-treated mice exhibited significant differences in processes related to acute inflammatory response, cytokine-mediated signalling, response to endoplasmic reticulum (ER) stress, protein folding, and regulation of lipid metabolism. Indeed, MaR1 downregulated the expression of genes involved in inflammation and ER stress (ie. Saa family and Xbp1) while upregulating genes involved in liver metabolic homeostasis and mitochondrial biogenesis (ie. Ppargc1a). Notably, isolated hepatic mitochondria from MaR1-treated mice showed a significant improvement in mitochondrial respiratory capacity. Overall, these results show that MaR1 alleviates hepatic metabolic dysfunction by dampening inflammatory and ER stress pathways while promoting mitochondrial function, supporting MaR1 therapeutic potential in aging and obesity related MASLD. Funding: MICIU/AEI/10.13039/501100011033 and ERDF, EU (PID2023-147572OB-I00, BFU2015-65937-R) and by CIBEROBN (CB12/03/30002).

Session 3

THE CRUCIAL ROLE OF PLATELET EICOSANOIDS IN CANCER: AN OPPORTUNITY TO PREVENT CANCER DEVELOPMENT AND METASTASIS THROUGH TARGETED THERAPEUTIC INTERVENTIONS

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Recent research has expanded the understanding of platelet functions beyond their conventional role in thrombosis. Platelets and their extracellular vesicles (EVs) can accumulate in inflamed tissues and, by interacting with numerous cell types, contribute to cancer and tumor metastasis. Platelets are a rich source of lipids derived from arachidonic acid (C20:4, n-6), including thromboxane A_2 (TXA_2) and 12(S)-hydroxy-5,8,10,14-eicosatetraenoic acid 12S-HETE) produced by the activity of the oxygenase enzymes cyclooxygenase-1 (COX-1), and 12-lipoxygenase (12-LOX), respectively. Evidence supports the notion that platelet activation, associated with TXA_2 biosynthesis, contributes to early carcinogenesis and metastatic progression. TXA_2 operates via two distinct mechanisms. Firstly, at sites of gastrointestinal mucosal lesions, it fosters a local inflammatory response characterized by COX-2 induction and increased prostaglandin (PG)E_2 biosynthesis, contributing to initial carcinogenic events. Secondly, TXA_2 binds to its receptor (TP) on T cells and activates ARHGEF1, a guanine exchange factor that converts inactive GDP-bound RhoA into its active GTP-bound form. The activation of RhoA inhibits T cell receptor-driven kinase pathways, T cell proliferation, and effector functions, ultimately suppressing anti-metastatic immunity. Consequently, the inhibition of platelet TXA_2 biosynthesis is considered a crucial mechanism underlying the anticancer effects of low-dose aspirin (75-100 mg/day). Aspirin irreversibly and selectively inhibits COX-1 activity in platelets, thereby reducing TXA_2-dependent platelet activation. Platelet-type lipoxygenase (pl12-LOX) catalyzes the production of the lipid mediator 12S-hydroperoxyeicosa-5,8,10,14-tetraenoic acid (12S-HpETE), which is rapidly reduced by cellular peroxidases to form 12S-HETE. Upon platelet activation, 12S-HETE is produced and esterified to membrane phospholipids, such as phosphatidylethanolamine and phosphatidylcholine, in considerable amounts, thereby promoting thrombin generation. Recent findings indicate that the crosstalk between platelets and cancer cells induces epithelial-mesenchymal transition (EMT) in cancer cells - an event associated with high-grade malignancy. These cancer cells subsequently produce 12-HETE, a modulator of cancer metastasis, which is predominantly esterified in plasmalogen phospholipids and is associated with changes in EMT gene expression. In conclusion, the discovery of new roles for platelet TXA_2, in cancer suggests novel uses for low-dose aspirin and other antiplatelet agents. However, these drugs can increase the risk of bleeding. New antiplatelet agents, such as 12-LOX inhibitors, are in clinical development to provide effective treatment with lower bleeding risk.

LOW-DOSE ASPIRIN FOR SECONDARY PREVENTION IN COLORECTAL CANCER: RESULTS FROM THE NORDIC ALASCCA TRIAL - A 50% REDUCTION IN RECURRENCE IN GENETICALLY SELECTED PATIENTS

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The ALASCCA trial represents a translational bridge between lipid mediator biology and modern biomarker-driven precision oncology. By targeting COX-dependent lipid signaling with aspirin in molecularly selected colorectal cancer, we demonstrated a substantial reduction in recurrence risk. Background Colorectal cancer (CRC) affects 1.9 million people globally each year, with 20–40% of stage II–III patients developing metastases. Observational studies suggest that aspirin can improve survival in patients with PIK3CA mutated, but not wild-type, CRC tumours. Randomized controlled trials are needed to confirm these findings. The ALASCCA (Adjuvant Low-dose Aspirin in Colorectal Cancer) trial was designed to prospectively evaluate the efficacy of adjuvant aspirin in patients with resected stage I–III CRC harboring somatic alterations in the PI3K signaling pathway. Methods The ALASCCA trial was a randomized, double-blind, multicentre, placebo-controlled trial in patients in the Nordic countries with stage II-III colon cancer or stage I-III rectal cancer with somatic alterations in the PI3K-pathway. Patients were screened for tumour somatic alterations in PIK3CA exon 9/20 hotspots (Group A), and PIK3R1, PTEN, or other PIK3CA alterations and randomized 1:1 post-surgery to receive 160 mg of aspirin or placebo once daily for three years. The primary endpoint was time to local recurrence or distant metastases (TTR) in Group A, analysed using a Cox proportional hazards model. Results In total, 3,508 patients were screened for somatic alterations, of which 2,980 had complete genomic data, with PI3K-altered tumours in 1,103 patients (37%). Of these, 626 patients were randomized; 314 in Group A, and 312 in Group B. The hazard ratio (HR) for TTR was 0.49 (0.24-0.98; p=0.044) in Group A, and 0.42 (95% CI 0.29-0.88; p=0.017) in Group B. HRs for DFS in Group A and B were 0.61 (95% CI 0.34-1.08; p=0.091) and 0.51 (0.29-0.88; p=0.017). Four patients experienced aspirin-related severe adverse events. Conclusion Adjuvant aspirin compared to placebo reduced recurrence rate in patients with PI3K-altered CRC. These findings offer a safe, globally available, and cheap treatment option that can change clinical practice for around a third of patients with early-stage CRC.

RELIEVING PROSTAGLANDIN-MEDIATED IMMUNOSUPPRESSION UNLOCKS NEOANTIGEN VACCINE EFFICACY

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Personalized cancer vaccines targeting tumor-specific neoantigens show great promise, but their effectiveness in solid tumors is often limited by immunosuppressive factors in the tumor microenvironment. Prostaglandin E_2 (PGE_2) is a key lipid mediator that contributes to immunosuppression in the tumor microenvironment, and selective inhibition of microsomal prostaglandin E synthase 1 (mPGES-1) provides a way to suppress PGE_2 production without the cardiovascular adverse effects associated with cyclooxygenase inhibition. We investigated whether modulating prostaglandin signaling could enhance neoantigen-driven immunotherapy in the MC38 tumor model. A customizable neoantigen-coupled bead vaccine, manufactured for MC38-specific neoantigens, was combined with a selective mPGES-1 inhibitor and evaluated for effects on tumor growth, lipid mediator profiles, and intratumoral immune composition. Neither treatment alone was sufficient to restrain tumor progression; however, combined administration significantly delayed tumor growth. This therapeutic effect was associated with a shift in the intratumoral prostaglandin balance, consistent with effective mPGES-1 target engagement. Flow cytometry analysis showed that combination treatment increased infiltration of $CD8^+$ T cells and reshaped the exhaustion landscape within the tumor. Specifically, tumors from combination-treated animals exhibited an increased frequency of precursor exhausted $CD8^+$ T cells and a reduction in terminally exhausted $CD8^+$ T cells, indicating a more functional and therapeutically responsive T cell compartment. Maximal efficacy required concurrent administration of the mPGES-1 inhibitor with the neoantigen vaccine. These findings highlight the interplay between lipid mediators and anti-tumor T cell responses and support targeting mPGES-1 as a complementary strategy to improve neoantigen-based immunotherapy in solid tumors.

ROLE OF MPGES-1 IN IRINOTECAN-INDUCED GASTROINTESTINAL TOXICITY

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Irinotecan (CPT-11), a camptothecin derivative, is a potent chemotherapeutic agent used for various cancers. Despite its efficacy, its clinical utility is severely hampered by significant unpredictable adverse effects, most notably myelosuppression and debilitating diarrhea. These toxicities often necessitate dose reductions, compromising treatment outcomes. Previous studies have indicated that CPT-11 induces COX-2 expression and PGE_2 production in colon. Furthermore, co-administration of CPT-11 and celecoxib (a selective COX-2 inhibitor) enhances the anti-tumor efficacy of CPT-11 while attenuating diarrhea. These findings suggest that COX-2/PGE_2 plays a critical role in the development of CPT-11-induced diarrhea. Microsomal PGE (mPGES)-1 is the terminal enzyme that preferentially couples with COX-2 to generate PGE_2. While mPGES-1 is typically associated with the progression of inflammatory diseases and cancer progression, its specific contribution to CPT-11-induced intestinal toxicity remained unclear. In this study, we examined the role of mPGES-1 in this model using mPGES-1-deficient mice. Contrary to our hypothesis, CPT-11-induced diarrhea and intestinal mucosal damage were aggravated in mPGES-1-deficient mice. Notably, this exacerbated toxicity was significantly attenuated by the co-administration of either butaprost (an EP2 receptor agonist) or CAY10598 (an EP4 receptor agonist). These results suggest that mPGES-1-derived PGE_2 plays a crucial role in maintaining the integrity of the gastrointestinal tract against CPT-11-induced damage via the activation of EP2 or EP4 receptors.

Plenary lecture

THE ENDOCANNABINOIDOME, THE GUT MICROBIOME AND DIETARY FATTY ACIDS: A WELL FUNCTIONING TRIANGLE

Vincenzo Di Marzo

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The two arachidonic acid-derived endogenous ligands of cannabinoid receptors, the endocannabinoids anandamide and 2-arachidonoyl-glycerol, are only the tip of an iceberg of a larger signaling system composed of hundreds of chemically similar long chain fatty acid derivatives, including 2-monoacyl-glycerols, N-acyl-ethanolamines and other N-acyl-amines. Unlike the endocannabinoids, these lipids only weakly bind the CB1 and CB2 cannabinoid receptors, and modulate instead the activity of other G protein-coupled receptors, as well as of ligand-activated ion channels and nuclear receptors, which regulate energy metabolism, the immune/inflammatory response and behaviour, among others, in mammals. Bacteria of the gut microbiome also produce endocannabinoid-like molecules capable of activating these same receptors. These discoveries led to reveal the existence of bi-directional communications between this "extended endocannabinoid system", or endocannabinoidome, and the gut microbiome, under the control of dietary fats. I will discuss the role of this triangle with particular emphasis on metabolic inflammatory and affective disorders.

Session 4

INTERACTION BETWEEN LIPIDS AND GUT MICROBIOTA INFLUENCES METABOLIC HEALTH

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My research focuses on understanding how dietary lipids interact with the gut microbiota to regulate metabolic health and inflammatory processes. Although fatty acids are recognized as important signaling molecules that influence immunity, metabolism, and tissue function, their biological effects are strongly modified by the intestinal microbial community. A central goal of my work has been to define the mechanisms through which host–microbe interactions shape lipid-mediated signaling and contribute to metabolic disease. Using experimental models and translational approaches, we have investigated how gut microbes influence host responses to dietary fatty acids and how these interactions affect inflammatory pathways, adipose tissue function, hepatic lipid metabolism, and systemic metabolic homeostasis. Our findings demonstrate that the metabolic consequences of dietary lipids are determined not only by lipid structure and composition, but also by microbial-dependent processes that regulate lipid sensing, signaling, and metabolism. These interactions influence the development of chronic low-grade inflammation, hepatic steatosis, dyslipidemia, and insulin resistance. In this presentation, I will discuss how gut microbiota–lipid interactions shape metabolic and inflammatory responses, highlighting emerging concepts on the role of microbial modulation of fatty acid signaling in obesity-related metabolic disorders and liver disease.

INFLAMMATION RESOLUTION IN RETINAL DEGENERATION: THE EMERGING ROLE OF SPECIALIZED PRO-RESOLVING LIPID MEDIATORS IN HEREDITARY RETINAL DISEASES

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Background: Failure to resolve inflammation, rather than excessive inflammation alone, is increasingly recognized as a key driver of chronic degenerative diseases. Specialized pro-resolving lipid mediators (SPMs), derived from omega-3 fatty acids, actively orchestrate the termination of inflammation and restoration of tissue homeostasis. Their contribution to hereditary retinal diseases remains largely unexplored. Purpose: To investigate whether impaired SPM-driven resolution contributes to retinal degeneration and to evaluate the therapeutic potential of targeting this pathway. Methods: rd10 mice were orally supplemented with SPM-enriched marine oil. Retinal structure and function were assessed by histology and electroretinography. Microglial responses were characterized by immunohistochemistry and gene expression profiling, focusing on pro-inflammatory and pro-resolving activation programs. In parallel, exploratory translational analyses were conducted in samples from patients with inherited retinal diseases to assess alterations in SPM biosynthesis and signaling pathways. Results: SPM supplementation significantly preserved retinal integrity and improved visual function in rd10 mice. These effects were associated with a shift of retinal immune responses toward a pro-resolving state, accompanied by attenuation of inflammatory signaling. Importantly, translational analyses in patient-derived samples revealed indications of an altered SPM landscape, suggesting impaired endogenous resolution capacity in human disease. Conclusions: These findings support a paradigm shift in retinal degeneration, identifying defective inflammation resolution as a critical and targetable mechanism. Enhancing SPM pathways, rather than broadly suppressing inflammation, emerges as a promising strategy to restore immune homeostasis and limit neurodegeneration. Funding: This work was supported by Instituto de Salud Carlos III (ISCIII) (PI18/00252, PI22/00082, PI25/00009), co-funded by the European Union).

Reference: Olivares-González L, Velasco S, Gallego I, Esteban-Medina M, Puras G, Loucera C, Martínez-Romero A, Peña-Chilet M, Pedraz JL, Rodrigo R. An SPM-enriched marine oil supplement shifted microglia polarization toward M2.

BEYOND DHA: KEY FATTY ACIDS DRIVING RETINAL DEGENERATION AND THEIR IMPLICATIONS FOR NUTRITIONAL THERAPIES

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Retinal diseases are characterized by common disruptions in homeostasis, including inflammation, oxidative stress, endoplasmic reticulum stress, and altered phagocytic activity. These processes are tightly regulated by complex signalling networks. The retina is specially enriched in fatty acids (FAs), whose composition directly influences neuronal function. Roles of FAs go further than participating in membrane structure and energy supply since they can regulate metabolism and participate in inflammatory and apoptotic routes. Reduced levels of docosahexaenoic acid (DHA) have been reported in blood and neural tissues of patients and animal models with retinal dystrophies. Although numerous clinical trials have explored omega-3 FA supplementation, the outcomes have not fully met expectations. Notably, these studies focused mainly on DHA, while changes in other FAs have been largely overlooked. Here, we analysed the retinal fatty acid profile of the rd10 murine model of retinitis pigmentosa using GC/MS. The aim was to identify key FAs in the retina associated with the progression of the retinal disease and to uncover potential targets for nutritional intervention. Photoreceptor degeneration in retinitis pigmentosa mice was associated with a broad decline in specific retinal FAs. We observed a reduction in multiple short- and long-chain saturated FAs, as well as in several monounsaturated FAs, in the retinas of rd10 mice. In addition, levels of the n-6 polyunsaturated FA arachidonic acid and DHA were markedly diminished in the dystrophic retina. Notably, the decrease in DHA was more pronounced, resulting in a significant increase in the n-6/n-3 ratio under degenerative conditions, indicative of a pro-inflammatory state. Interestingly, myristic acid was markedly reduced, suggesting that the interaction of proteins of the phototransduction cascade with lipid membranes may be affected in this model, and representing an interesting target for therapy. Our findings challenge the current DHA-centric paradigm and highlight that retinal degeneration is accompanied by a complex, disease-specific FA signature. This work opens new avenues for the development of next-generation dietary lipid interventions, moving toward more precise and effective neuroprotective strategies.

LOW DOSE N-3 PUFA SUPPLEMENTATION SHIFTS PLASMA OXYLIPINS FROM AA- TO EPA-DERIVED PATHWAYS: A SUB-STUDY OF THE GISSI-PREVENZIONE TRIAL

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Background: Supplementation with n-3 polyunsaturated fatty acids (n-3 PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been associated with cardiovascular benefits. However, the results from many clinical trials remain inconsistent. To better understand the effects of supplementation, we determined the response in circulatory oxylipin profiles to 12-month n-3 PUFA supplementation (850 mg/day, EPA:DHA ≈ 1:2) in post-myocardial infarction (MI) patients from the GISSI-Prevenzione trial. Methods: 80 participants (n=40 standard-of-care controls, n=40 supplemented) were selected to achieve a balance in terms of age, sex, BMI, smoking status, and baseline clinical parameters. Plasma was collected at baseline and 12-month follow-up. Of 141 oxylipins screened by targeted LC-MS/MS, 90 were above the limit of quantification with >50% detection rate per group. The observed oxylipins were primarily derived from arachidonic acid (AA), EPA, and DHA via cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) activity. Analysis was performed at pathway level or individual metabolite. Pathways were predefined by precursors or primary enzymes. Relative changes of pathways were calculated as mean Δlog (Concentration) Pathway A-mean Δlog (Concentration) Pathway B, where Δlog was the within-subject difference between follow-up and baseline. The r (effect size) was determined by a Wilcoxon test. Results: Supplementation with 1g of n-3 PUFA produced a broad increase in EPA-derived oxylipins, including monohydroxys (5-, 8-, 9-, 11-, and 15-HEPE; $q<0.10$), CYP450-derived dihydroxys (14,15- and 17,18-DiHETE; $q<0.10$), and specialized pro-resolving mediators (SPMs) (18-HEPE, RvE2, and RvE4; $q<0.10$). In contrast, no major effect was observed in DHA-derived oxylipins despite higher formulation content, except for the CYP450 product 19,20-DiHDoPE, which increased significantly ($q<0.10$). All four CYP450-derived AA dihydroxys (5,6-, 8,9-, 11,12-, and 14,15-DiHETrE) increased over time in controls ($q<0.10$); however, there was no increase in the supplemented group. For each enzymatic pathway, the relative EPA-to-AA difference yielded significant between-group effects (n-3 PUFA vs control): CYP450 ($r=0.45$, $q<0.01$), LOX ($r=0.40$, $q<0.01$), and COX ($r=0.35$, $q<0.05$). Conclusions: Supplementation of 1g n-3 PUFA for 12 months in post-MI patients shifts oxylipin biosynthesis from AA- toward EPA-derived pathways across LOX, COX, and CYP450. CYP450 epoxygenase activity showed the greatest responsiveness, providing downstream molecular insight into the biological impact of n-3 PUFA supplementation.

Third Sponsored Session

Resolution of Inflammation
Sponsored by COST EU Resolve

RESOLUTION OF INFLAMMATION; STATE-OF-THE-ART AND TRANSLATIONAL LANDSCAPE

Mauro Perretti

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The term ‘Resolution Pharmacology’ refers to the possibility of exploiting the fundamental biology of the resolution of inflammation to guide the development of novel therapeutics. Here we summarise some of the efforts which have been made to develop pro-resolving molecules. The resolution phase of inflammation is effected through the actions of several mediators which act in concert and regulate each other in a fully integrated fashion. The observation that the inflammatory reaction that our body mounts always, or nearly always, resolves indicates that inflammation resolution is a robust process. What remains to be addressed, though, is how to harness the biology of acute resolving inflammation so that innovative therapeutic options can be offered for the clinical management of chronic non-resolving inflammation. In this presentation we use Annexin A1 as a non-lipid pro-resolving mediators, and a paradigm to aid the development of novel therapies. We remain of the opinion that Resolution Pharmacology remains an untapped opportunity for the pharmaceutical industry as well as for the benefit of patients affected from chronic diseases.

ROLE OF SPECIALIZED PRORESOLVING MEDIATORS IN CARDIOVASCULAR DAMAGE IN HYPERTENSION

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Excessive local inflammation is a common mechanism in many cardiovascular diseases (CVDs) such as hypertension. This is driven by the infiltration of proinflammatory immune cells that release cytokines such as interferons, tumour necrosis factor alpha or interleukins that increase oxidative stress and contractile prostanoids and favour the expression of adhesion molecules that further allow leucocytes recruitment and reduce nitric oxide (NO) availability and other protective factors, thereby impairing cardiovascular function. Resolution of inflammation is mediated by a family of specialized pro-resolving mediators (SPMs) generated from omega-3 and omega-6 polyunsaturated fatty acids: lipoxins, resolvins, protectins and maresins. These SPMs act on cognate G protein-coupled receptors to limit immune cell infiltration and initiate tissue repair. SPM receptors are expressed in immune and cardiovascular cells where they regulate important processes such as phagocytosis and polarization, production of cytokines, NO and prostacyclin, and modulation of cell phenotype. Different SPMs have shown beneficial effects in cardiovascular function and structure in various CVD. I will show recent advances in the role of inflammation and SPMs in cardiovascular (dys)function in hypertension.

Session 5

CLINICAL TRIALS OF OMEGA 3 FATTY ACIDS FOR CARDIOVASCULAR PREVENTION

Philippe Gabriel Steg, Jules MESNIER

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In the past 20 years, multiple clinical trials have explored the cardiovascular benefit of omega 3 fatty acids in secondary and primary prevention. These trials have used a variety of omega 3 fatty acids formulations and doses, in diverse patient populations and have yielded apparently inconsistent results. Specifically, several trials using relatively high dose EPA or EPA:DHA (JELIS, REDUCE IT, RESPECT EPA, and, more recently PISCES) have found clinical cardiovascular benefits. This presentation will attempt to clarify the trial results and the potential explanations for apparent discrepancies across trials, as well as describe potential benefits and side effects of omega 3 fatty acids.

RESOLVING CARDIOVASCULAR INFLAMMATION THROUGH OMEGA-3-DERIVED LIPID MEDIATORS

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Immune activation plays a central role in cardiovascular disease, and its balance with timely resolution determines whether inflammation remains protective or becomes pathological for the cardiovascular system. Failure to resolve inflammation is a common feature across cardiovascular diseases, including atherosclerosis, myocardial infarction, heart failure, and valvular heart disease. Anti-inflammatory treatment options are emerging but may be associated with immunosuppression and increased inflammation susceptibility. Specialized pro-resolving mediators (SPMs), derived from fatty acids, actively terminate immune activation and promote tissue repair. Clinical and translational studies demonstrate dynamic regulation of SPMs, with early activation following myocardial infarction, and that an impaired resolution causes maladaptive myocardial remodelling and vascular dysfunction. Emerging evidence also highlights direct effects of SPMs on cardiac, thrombotic, and vascular functions. Taken together, these findings position inflammation-resolution as a therapeutic frontier extending beyond traditional anti-inflammatory strategies.

PROSTACYCLIN IS AN ENDOGENOUS PROTECTION AGAINST AORTIC STENOSIS PROGRESSION AND AORTIC VALVE PROSTHESIS DETERIORATION

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Aortic stenosis (AS) is the most common valvular heart disease. AS is characterized by a progressive calcification and thus a narrowing of the aortic valve (AV), leading to restricted blood flow, heart failure, and death if untreated. To date there is no treatment for AS but replacement with prosthetic valves. Lesions on the prosthesis surface occur, however, in many patients after successful aortic valve replacement, an obstacle called hypo-attenuated leaflet thickening (HALT). HALT may deteriorate AV prostheses, reduce their functional durability, or cause life-threatening thromboembolic events. The underlying mechanism of HALT is still unknown, but thrombosis is likely involved. Thrombosis is also a known factor that drives AS progression. We have elucidated a mechanism that can explain both AS pathogenesis in AV tissue and HALT formation on AV prosthesis. Using expression analysis, prostanoid profiling by LC-MS/MS, and functional thrombosis assays, we show that valvular interstitial cells (VIC) abundantly produce prostacyclin (PGI_2) as an endogenous anti-aggregant, which protects the tissue from microthrombus deposition. PGI_2 biosynthesis is gradually lost as VIC transform and AV tissue calcifies during the disease process, leading to increased thrombus deposition. Activated platelets in the thrombi promote an osteogenic program in VIC that aggravates AS progression. In AV prostheses, absent PGI_2 production leads to HALT formation and prosthesis deterioration. The COX-2–PGIS–IP axis may thus represent a promising target for a medical treatment to impede progression of AS.

INCREASED LIPID DYSHOMEOSTASIS IN METABOLIC SYNDROME DUE TO DELETION OF RESOLVIN D2 RECEPTOR GPR18 IN MICE

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Background and objective: Metabolic syndrome (MS), characterized by cardiovascular risk factors including abdominal obesity, impaired glucose metabolism, and dyslipidemia, is often associated with fatty liver disease. Increased steatosis and hepatic fibrosis is observed following myeloid-specific deletion of the G-protein-coupled receptor (GPR)18, which is the receptor for the specialized pro-resolving lipid mediator, resolvin D2 (RvD2). The objective of this study was to investigate the effect of GPR18 deletion on the development of MS in mice. Methods: Adult male adult wild-type (WT) and GPR18 knock-out (KO) mice were placed on a high fat (45% kcal)/high fructose (30%) (HF/HF) diet for 16 weeks. Measurements included: body mass, plasma glucose, plasma and liver lipidomics analysis, and plasma lipoprotein profiles (UHPLC). Liver steatosis (hematoxylin/eosin) and fibrosis (Sirius red) were assessed by histology. Real-time qPCR was used for gene expression analysis. Results: Increased body mass, blood glucose, and plasma total cholesterol in both WT and KO mice, as well as steatosis were observed, confirming MS development. Although plasma and liver cholesterol were increased in both WT and KO mice on HF/HF diet, there was a significantly higher level of HF/HF-induced plasma cholesterol levels in male KO as compared to WT mice, associated with the appearance of plasma LDL. In addition, qPCR analysis showed, only in KO mice on HF/HF diet, decreased expression of hepatic lipoprotein receptors, including the LDL-R, and the lipolysis stimulated lipoprotein receptor, LSR, as well as increased PPARγ expression. Furthermore, lipidomics analysis showed higher levels of hepatic lipids involved in lipid storage in KO as compared to WT mice, consistent with PPARγ's role in lipid storage regulation. Conclusions: Taken together, these results suggest that the absence of GPR18 exacerbates lipid dyshomeostasis in MS, which may be due to dysregulation of hepatic lipoprotein clearance and lipid storage. This work was supported by the B4B interdisciplinary program as part of the Lorraine Initiative of Excellence funded by France 2030, the EU Care-in-Health project, and the French MESRE.

DELETION OF GPR18 IN MICE LEADS TO ALTERED VASCULAR MECHANICAL PROPERTIES

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An emerging risk factor for cardiovascular disease (CVD) is the presence of unresolved chronic vascular inflammation. We recently demonstrated that the specialized pro-resolving lipid mediator, RvD2 contributes to resolution of inflammation and reducing atherosclerosis plaque in the vascular wall, presumably via its receptor, the G protein-coupled receptor (GPR)18. Here, our goal was to determine how the absence of the RvD2/GPR18 axis could affect vascular stiffness and cardiac function in GPR18 knock-out (KO) mice in basal conditions or following induction of the metabolic syndrome, a known risk factor for cardiovascular disease. Methods: Adult male and female wild-type (WT) and GPR18 knock-out (KO) mice were placed on a high fat (45% kcal)/high fructose (30%) (HF/HF) diet for 16 weeks. Body weight and metabolic parameters (glycemia, lipemia) were monitored, as well as the level of steatosis/fibrosis in the liver. Echocardiography was performed at T0 and T15 weeks. At the end of the experimental period, echotracking at T16 weeks was done before animals were sacrificed. Blood, and tissues including the aorta were removed for histological analysis. Results: Globally, echocardiography did not reveal any major modification. A decreased distensibility in thoracic aorta from GPR18 KO mice compared to wild type mice was detected. This modification was associated with decreased mean arterial pressure in female GPR18-KO mice on HF/HF diet as compared to GPR18 WT on the same diet. A higher content in collagen I in the media together with a lower level of the contractile marker sm-MHC were found in GPR18 KO mice by immunofluorescence, suggesting a dedifferentiation of smooth muscle cells following GPR18 deletion. Conclusions: Taken together, our results showed that the absence of GPR18 modifies the mechanical properties of the arterial wall causing decreased distensibility associated with dedifferentiated vascular smooth muscle cells. This work was supported by the EU Care-in-Health project, and by the French MESRI.

POSTER PRESENTATIONS

POSTER 1

INVESTIGATING THE ROLE OF a/B-HYDROLASE DOMAIN 12 (ABHD12) ON PROLIFERATION OF SELECTED BREAST CANCER CELL LINES

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Breast cancer remains the most prevalent malignancy among women globally, with dysregulated lipid metabolism recognised as a key hallmark of its progression. The lipid hydrolase ABHD12 regulates bioactive lipid signalling molecules, including endocannabinoids and lysophospholipids, which influence tumour proliferation, immune evasion, and metastasis. This study aimed to characterise ABHD12 enzymatic activity across breast cancer cell lines using activity-based protein profiling (ABPP) with fluorescent probes (FP-rhodamine and MB064), and to assess the inhibitory and anti-proliferation effects of the selective ABHD12 inhibitor DO264 in cancer cells. Optimised assay conditions were established at 37°C and 90 min incubation, yielding maximal probe labelling efficiency. Under these parameters, DO264 effectively and selectively inhibited ABHD12 activity in both rat brain particulate fraction and breast cancer cell lysates, validating the probe-inhibitor system's selectivity. DO264 inhibition was concentration-dependent in MDA-MB-231 cells but less pronounced in MCF-7, likely due to differential enzyme expression. A secondary ~41 kDa protein, tentatively identified as ACOT7, was also inhibited by DO264 in MDA-MB-231 cell preparations, suggesting potential off-target effects requiring proteomic confirmation. MTT assays revealed that DO264 reduced cell viability in both MCF-7 and MDA-MB-231 lines (IC50 ≈ 1 μM and ≈ 5 μM, respectively), while doxorubicin exhibited greater potency in the MTT assay but did not affect ABHD12 activity in the ABPP assay. Collectively, these results validate MB064 as a useful probe to detect ABHD12, confirm DO264 as a potent and relatively selective inhibitor, and highlight the context-dependent modulation of ABHD12 across tissue and cancer models. Future investigations will examine whether ABHD12 inhibition modulates doxorubicin efficacy in the MTT assay and whether it assists in assessing metastatic behaviour using a cellular migration assay. Moreover, literature descriptions of ABHD12 protein expression levels suggest that additional breast cancer subtypes with distinct profiles, such as Luminal B (BT-474) and HER2+ (SK-BR-3), could be examined, thereby providing a wider understanding of lipid hydrolase pathways in breast cancer progression and therapeutic potential.

POSTER 2

ARACHIDONIC ACID RESTORES ENDOCANNABINOID AND HIPPOCAMPAL LIPID-RELATED SIGNALING FOLLOWING CHRONIC CHLORPYRIFOS EXPOSURE UNDER PREDIABETIC CONDITIONS

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Introduction: Chronic exposure to organophosphate pesticides (OPPs), particularly chlorpyrifos (CPF), has emerged as a contributor to metabolic dysfunction, neuroinflammation, and cognitive decline. Increasing evidence indicates that disruption of lipid mediator pathways, including arachidonic acid (AA) metabolism and endocannabinoid signaling, may represent a mechanistic link between environmental toxicant exposure and neurodegeneration. Prediabetes further amplifies these disturbances through insulin resistance, oxidative stress, and impaired neuronal plasticity. However, the impact of chronic CPF exposure on hippocampal lipid-associated signaling under metabolically compromised conditions remains poorly understood. Aim: This study investigated the interplay between chronic CPF exposure, prediabetes, and dysregulation of AA/endocannabinoid pathways, while evaluating the potential protective effects of AA supplementation against neuro-metabolic dysfunction. Methodology: Sixty male Sprague–Dawley rats were allocated to control or prediabetic dietary regimens. Animals were dermally exposed to CPF (40% dermal LD_{50}) with or without oral AA supplementation (3 mg/kg/day). Cognitive and motor performance were assessed using novel object recognition, Y-maze, and pole tests. Cerebral and mesenteric perfusion were evaluated by laser speckle imaging. Biochemical analyses included glycemic indices, oxidative stress markers, inflammatory cytokines, cholinesterase activity, CB1 and CB2 receptors, and FAAH and MAGL enzymes hippocampal expression. LC–MS/MS quantified CPF, AA, anandamide (AEA), and 2-arachidonoylglycerol (2-AG). Hippocampal proteomic profiling was performed using high-resolution LC–MS/MS, followed by pathway enrichment analyses. Results: CPF exposure induced cognitive impairment, metabolic dysfunction, oxidative stress, and neuroinflammation, with substantially greater severity in prediabetic animals. Hippocampal proteomics revealed marked dysregulation of pathways related to oxidative phosphorylation, synaptic vesicle cycling, neurite branching, and insulin signaling. Notably, CPF and prediabetes shared 97 commonly altered proteins, indicating convergent neurotoxic mechanisms. CPF also altered endocannabinoid tone through elevated 2-AG levels, reduced CB1R, FAAH, and MAGL expression, and increased CB2R expression, accompanied by reduced serum AA levels. AA supplementation improved behavioral outcomes, attenuated inflammatory and oxidative markers, partially restored mitochondrial and synaptic proteins, normalized cannabinoid receptor expression, and mitigated neuronal degeneration. Conclusion: Chronic CPF exposure disrupts lipid mediator homeostasis and hippocampal metabolic signaling, particularly under prediabetic conditions. AA supplementation partially restores endocannabinoid balance and neuroprotective pathways, supporting its therapeutic potential as a lipid-based intervention against pesticide-induced neuro-metabolic dysfunction.

POSTER 3

CONTRACTILE INFLUENCE OF THE ENDOTHELIUM ON PROSTACYCLIN ANALOGUE/ MIMETIC INDUCED RELAXATIONS IN HUMAN PULMONARY ARTERIES

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Background and aim of the study: Pulmonary hypertension (PH) is a progressive and severe disease, particularly within Group-3 (secondary to a lung disease), where five-year survival rates can be as low as 31%. The beneficial vasorelaxant effect of drugs like prostacyclin (PGI_2) analogues (iloprost)/ mimetics (MRE-269), have been extensively studied in human pulmonary artery (HPA) smooth muscle (Norel et al., 2020). For this reason, the present study investigates the specific roles of these drugs on the HPA endothelium and the consequences on the muscular tone. Methods: HPA rings are set up in an organ bath system, they were pre-incubated (30 min) with or without pharmacological selective inhibitors like: a cyclooxygenase inhibitor (indomethacin 1.7 µM) or a NO-synthase inhibitor (L-NOARG 0.1 mM) or a HSP90 inhibitor (geldanamycin 1uM). After a pre-contraction produced by 10 µM of norepinephrine, dose-dependent relaxations to the PGI2 analogue/mimetic were induced (relaxations are expressed as % of the norepinephrine pre-contraction). Molecular mechanisms are further explored with Western blot analysis and ELISA to evaluate the involvement of PGI2 and NO signalling pathways. Results - Discussion: Greater maximal relaxations induced by MRE-269 (-129 +- 36%, n=5 / -77 +- 15%, n=5) and iloprost (-106 +- 9%, n=7 / -78 +- 9%, n=7) were measured in (absence / presence) of endothelium, respectively. This result for MRE69 is not in accordance with a previous work on HPA (Fuchikami et al., 2017). That could be explained by a contractile role of the endothelial cells, where endothelin synthesis/release from the endothelium could be a hypothesis. Moreover, indomethacin, but not L-NOARG, significantly enhanced iloprost-induced relaxations in endothelium-intact arteries, suggesting the involvement of endothelium-derived vasoconstrictor prostanoids. Conclusion: By characterizing these interactions, this research uncovers a new contractile effect of these PGI_2 analogues/mimetics that is dependent on the HPA endothelial layer. Ultimately, fully understanding these mechanisms will support the development of improved therapeutic strategies.

Reference: Norel et al., *Pharmacol.Rev.* 2020; 72(4):910-968. PMID: 32962984 Fuchikami et al., *Eur J Pharmacol.* 2017; 795:75-83. PMID: 27919660.

POSTER 4

TNF EXPRESSION AND DHA CONTENT RULE THE SENSITIVITY TO FERROPTOSIS OF LPS-ACTIVATED RAW264.7 MACROPHAGES

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Ferroptosis is a special type of cell death that is driven by lipid peroxidation by Fenton-like reactions. By inducing cell death in inflammatory cells, ferroptosis may play a key role in the regulation of inflammation. However, the relationship between lipid mediator metabolism and the sensitivity to ferroptosis of inflammatory cells is not completely understood. To understand the role of the precursors of lipid mediators in the sensitivity to ferroptosis of activated macrophages, we treated RAW264.7 cells with lipopolysaccharide 30 ng/mL. Subsequently we treated the macrophages with arachidonic acid (AA, 2 and 10 μM), docosahexaenoic acid (DHA, 2 and 10 μM), or acetylsalicylic acid (ASA, 10 and 100 μM). To determine the sensitivity to ferroptosis, we treated the cells with RSL3 7.5 μM and the calculated the ratio of survivorship. In addition, we determined by qPCR the gene expression of different genes related with ferroptosis (transferrin receptor), inflammation (Tnf, Il18, Il1b, Pparg, Mrc1), and endoplasmic reticulum stress (Hspa5). For all treatments, LPS-activated macrophages presented stronger resistance to ferroptosis than resting macrophages. Among resting macrophages, we found that AA in concentration 2 μM provoked the strongest increase in sensitivity to ferroptosis. In contrast, among LPS-activated macrophages, DHA 10 μM induced the strongest increase in sensitivity to ferroptosis. To understand these changes, we performed a Bayesian multivariate regression on the sensitivity to ferroptosis. We used gene expression and the type of treatment (AA, DHA, ASA) as predictors. The multivariate regression showed that, all other equal, the higher the expression of Tnf, the lowest the sensitivity to ferroptosis. In addition, when the effect of gene expression was taken into account by the regression, DHA 10 μM coefficient presented the strongest sensitization to ferroptosis. Our results show a specific regulation of the sensitivity to ferroptosis in LPS-activated macrophages. In relation to the precursors of lipid mediators, our results suggests that a combined treatment reducing Tnf expression and exposing to DHA might sensitize activated macrophages to ferroptosis. The sensitization of activated macrophages to ferroptosis may dampen macrophage-associated inflammation.

POSTER 5

TARGETING THE G-PROTEIN COUPLED RECEPTOR 183 IN THE CONTEXT OF MULTIPLE SCLEROSIS.

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Multiple Sclerosis (MS) is characterized by immune cell infiltration into the central nervous system (CNS), chronic inflammation, and demyelination. Among emerging therapeutic strategies, bioactive lipids, including oxysterols, oxidized derivatives of cholesterol, have gained increasing attention due to their roles in immune regulation. Among them, 7α,25-dihydroxycholesterol (7α,25-diOHC), produced by cholesterol-25-hydroxylase (CH25H) and CYP7B1, acts as a potent chemotactic factor. By binding to the G protein-coupled receptor GPR183 (EBI2), it regulates the migration of immune cells, including Th17 lymphocytes, toward the CNS. The 7α,25-diOHC - GPR183 axis has been implicated in immune cell chemotaxis in several inflammatory and autoimmune diseases. Evidence from murine knockout models supports its involvement in MS pathogenesis. It has been shown to promote the migration of autoreactive lymphocytes into the CNS, contributing to inflammatory processes, while also participating in the recruitment of oligodendrocyte progenitor cells to demyelinated areas, suggesting a potential role in remyelination. In this context, our work aimed to evaluate the therapeutic potential of pharmacological blockade of the 7α,25-diOHC - GPR183 axis in preclinical MS models. We first examined the impact of the disease on the expression of CH25H, CYP7B1, and GPR183. Then, we assessed the effects of GPR183 antagonism using NIBR189 and “compound 32” (Jianbei Xi. et al, 2023) in two complementary vivo models, namely the experimental autoimmune encephalomyelitis (EAE), to investigate immune cell infiltration and inflammation, and the cuprizone-induced demyelination model, to evaluate remyelination processes. The results obtained reveal a global upregulation of this pathway in vitro in activated glial cell cultures, as well as in both in vivo models. Pharmacological blockade of GPR183 modulates key pathological parameters, supporting its involvement in disease progression. These findings suggest that the pharmacological targeting of this axis may represent a therapeutic strategy in MS by limiting immune cell infiltration while potentially influencing remyelination.

POSTER 6

HETES IN THE HEAT OF INFLAMMATION: LIPID MEDIATORS SHAPING PHAGOCYTE FUNCTION IN MS

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Lipid mediators derived from arachidonic acid are key regulators of innate immune signaling. However, their role in shaping phagocyte function during demyelination remains poorly understood. In multiple sclerosis (MS), myelin uptake by macrophages and microglia can drive both inflammatory and repair associated phenotypes. Here, we examine how hydroxyeicosatetraenoic acids (5-HETE, 11-HETE, and 15-HETE) contribute to this process. Using bone marrow–derived macrophages (BMDMs) and primary mouse microglia, we performed lipidomic analyses following stimulation with myelin and lipopolysaccharide (LPS). Preliminary results show that intracellular levels of 5-, 11-, and 15-HETE increase upon myelin exposure in a dose dependent manner. While myelin alone promotes intracellular accumulation, the secretion of these mediators into the extracellular environment remains limited. However, LPS stimulation induces their release from myelin-stimulated phagocytes, suggesting that inflammatory cues are required for mediator efflux. Currently, we are investigating how exogenous HETEs modulate phagocytes' inflammatory phenotype. Additionally, we make use of an ex vivo brain slice model to study how these lipid mediators influence remyelination. We hypothesize that 5-, 11-, and 15-HETE integrate myelin-derived and inflammatory signals, shaping phagocyte responses with potential implications for remyelination. By further unraveling the underlying pathways, we aim to uncover novel mechanisms that could be harnessed to enhance remyelination and open new therapeutic avenues for multiple sclerosis.

POSTER 7

CH25h/25-HC REGULATES ANGIOGENESIS DURING TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION.

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Ischemic stroke arises from occlusion of a cerebral artery, triggering irreversible tissue damage and persistent neurological deficits. Neuroinflammation, BBB disruption, and immune dysregulation represent core pathological features of stroke pathogenesis. Angiogenesis is a critical component of the repair response following ischemic stroke, contributing to tissue remodeling, restoration of perfusion, and functional recovery. In the peri-infarct region, hypoxia and inflammation trigger a coordinated cascade of molecular events, including the upregulation of pro angiogenic factors such as VEGF, angiopoietins, and HIFs. These signals promote endothelial cell proliferation, migration, and formation of new vascular networks. However, post stroke angiogenesis is often dysregulated, resulting in immature and leaky vessels that may compromise BBB integrity and exacerbate injury. Understanding the balance between beneficial and pathological angiogenic responses is therefore essential for developing therapeutic strategies aimed at enhancing vascular repair while minimizing adverse outcomes. This project identifies Ch25h and its product 25-hydroxycholesterol (25-HC) as a negative regulator of inflammation induced angiogenesis. We demonstrate that endothelial cell Ch25h deficiency is sufficient to enhance proliferation, whereas 25-HC treatment potently inhibits it. These findings were validated across both aortic ring models and primary brain blood barrier (BBB) endothelial cultures. These models together provide a physiologically accurate readout and a more representative genomic profile compared to standard cell lines commonly used in angiogenesis assays. Single cell RNA sequencing reanalysis and complementary experimental validation confirmed Ch25h expression in BBB endothelial cells 48 to 72 hours after transient middle cerebral artery occlusion. In mice with endothelial BBB specific deletion of Ch25h, we observed an increased vascular area in the striatum within the lesion 3 days after stroke. However, mice with Ch25h deletion in BBB tended to lose more weight and exhibited more leaky vessels as shown by IgG staining. Our data suggests that the Ch25h/25-HC axis acts as a brake on post ischemic angiogenesis and is necessary to structure non-leaky vessels during BBB angiogenesis.

POSTER 8

DISRUPTED LIPID METABOLISM AND INFLAMMATORY RESOLUTION PATHWAYS UNDERLIE NEUTROPHIL DEFECTS IN CYSTIC FIBROSIS

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Cystic fibrosis is characterized by persistent pulmonary inflammation that severely impairs respiratory function. Neutrophils dominate this inflammatory milieu and exhibit marked phenotypic and functional alterations, including dysregulated microbicidal activity, impaired apoptosis, and an increased proportion of immature, pro-inflammatory circulating cells. CFTR modulators have significantly improved disease management. Kaftrio, a triple combination therapy (Elexacaftor/Tezacaftor/Ivacaftor, ETI), provides major clinical benefits and reduces pulmonary inflammation. However, its specific impact on neutrophil biology remains poorly understood. Aims This study aims to characterize neutrophil phenotypic and functional dysregulations in cystic fibrosis and determine whether ETI treatment restores neutrophil homeostasis. Methods Blood samples were collected from cystic fibrosis patients before (V1) and after ETI treatment (V2), and from healthy donors (HD). The immune phenotype of neutrophils was analyzed using 24-marker spectral flow cytometry in whole blood. Neutrophils were isolated and processed for metabolomics and proteomics followed by mass spectrometry and integrated bioinformatic analyses (MetaboAnalyst, STRING, PANTHER, Ingenuity). Biological pathways were reconstructed by integrating enzymes with metabolites identified through metabolomics. Neutrophil apoptosis, a key step in inflammatory resolution, was assessed after 16h of culture by flow-cytometry. Results 4500 proteins and 120 metabolites were identified. Significant differences were observed between HD and V1 and were accentuated in V2. Comparisons between V1 and V2 revealed additional differentially expressed proteins and metabolites. Most dysregulated pathways involved lipid metabolism, triglyceride storage, immune suppression and pro-resolving mechanisms including resolvins. Key pro-resolving proteins (ALOX5, arginase-1, phospholipases, galectin-3, annexins) were altered, and PD-L1 expression was increased. These findings indicate profound metabolic reprogramming of neutrophils with enhanced fatty acid synthesis and storage, potentially impairing inflammatory resolution. Neutrophils from patients showed reduced apoptosis which was restored by ETI treatment. Conclusions These multi-omics analyses reveal key mechanisms of neutrophil dysfunction in cystic fibrosis and show that ETI partially restores altered pathways. In this context, lipid metabolism and apoptosis emerge as promising targets to improve inflammatory resolution.

POSTER 9

ANTI-INFLAMMATORY COMPOUNDS FROM *SALVIA MILTIORRHIZA* BGE. TARGET PROSTANOID PRODUCTION AND MPGES-1 ACTIVITY

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Rheumatoid arthritis (RA) is a chronic autoimmune disease that is often difficult to treat. Pro-inflammatory prostanoids like prostaglandin E2 (PGE2) have a central role in RA pathogenesis, and key enzymes of the arachidonic acid pathway like cyclooxygenase 2 (COX-2) or microsomal prostaglandin synthase 1 (mPGES-1) are targets for drug development. Our work aims to identify anti-inflammatory compounds from traditional Chinese medicinal plants and study their effects on prostanoid production to identify potential new drugs. Pure compounds isolated from a variety of Chinese medicinal plants were tested on different cell reporter assays for their ability to downregulate inflammatory signalling pathways (NFκB, STAT3, STAT5, NFAT) and prostanoid production. After screening 64 pure compounds, *Salvia miltiorrhiza* Bge. was the plant with the most compounds affecting prostanoid production. Finally, these compounds were evaluated by cell painting using the well-established cell line U2OS, as well as by the mPGES-1 enzymatic activity assay. Our data showed that 5 out of the nine compounds (cryptotanshinone, tanshinone I, dihydrotanshinone I, sugiol, danshexinkun A) isolated from this plant downregulated prostanoids (PGE2, TxB2, PGD2, PGF2a) produced by the synovial fibroblast cell line SW982, with the most potent one having an IC50 of 10 nM. Each compound also showed a distinct effect on the different signalling pathways tested. The cell painting phenotypic screening revealed similarities of the compounds with many known drugs, including cyclooxygenase inhibitors and mPGES-1 inhibitors. Specifically, cryptotanshinone showed the highest correlation with many known mPGES-1 inhibitors and this effect was further supported by the mPGES-1 activity assay where cryptotanshinone showed good inhibitory activity against mPGES-1.

POSTER 10

MULTI-STEP TOTAL SYNTHESIS OF PHENOLIC PHYTOPROSTANES

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Lipophenols, polyphenolic compounds acylated by a fatty acid, have recently been identified in food matrices naturally rich in both polyphenols and fatty acids, making them natural derivatives present in human diet. The identification of natural lipophenols is particularly relevant to understand their pharmacological actions, metabolism or to use them as analytical standards. As an example, hydroxytyrosol (HT) linked to polyunsaturated fatty acids (PUFA) is naturally present in extra virgin olive oil (EVOO) [1] and should participate to its antioxidant properties. As a preliminary work, the chemical synthesis of HT lipophenols allowed UHPLC-MS/MS quantitative study in EVOO during a 12 months period, mimicking both commercial and inappropriate conditions of storage. The results highlighted HT-OA as a relevant marker for the monitoring of oil storage conditions and quality. [2] Based on this study, an emphasis was put on HT-ALA, exhibiting a different analysis pattern than its analogues. This result might be due to oxidation of this compound to form phenolic phytoprostanes. Phytoprostanes (PhytoPs) are non-enzymatic lipid peroxidation products coming from ALA, biomarkers of oxidative stress in plants. This hypothesis was strengthened by the literature, showing that phytoprostanes coming from ALA were present in some vegetal oils,[3] as well as preliminary oxidation studies on HT-ALA in flask. The first stereoselective total synthesis of phenolic PhytoPs as analytical standards was therefore performed in 20 steps with a 3% global yield (84% average yield by step) from commercially available 1,3-cyclooctadiene. The lactol key intermediate was synthesized in 11 steps with controlled stereochemistry, which then allowed the introduction of the two side chains using Wittig and HWE reactions.

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POSTER 11

TOWARDS BROAD OXYLIPIN SCREENING IN TISSUE SAMPLES VIA TIMSTOF AND QQQ-MS

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Oxylipins, a class of bioactive lipids, have been shown to play key roles in modulating immune cell functions, notably affecting the outcomes of diseases such as cancer. Particularly, prostaglandin E2 (PGE2) is known to regulate a variety of immune cell functions, which includes limiting the expansion of tumor infiltrating T-cells (Lacher et.al 2024). However, many other oxylipins and their role in the regulation of tissue immunity are incompletely understood. Especially, their short-lived nature and therefore very localised effect makes a detection in tissue necessary.

To deepen the understanding in the role of oxylipins a broad analytical panel is needed, multiple of which have already been described (Chaves-Filho et al. 2023). Here we present the comparison of the performance between a timsTOF and a triple quadrupole (QQQ) mass spectrometers for the quantification of 52 oxylipins including prostaglandins, leukotrienes and thromboxanes, as well as evaluation of post-column modification of the LC flow. For the LC gradient, as well as for the MS parameters of the QQQ values were taken from the literature (Chaves-Filho et al. 2023). Meanwhile the MS parameters on the timsTOF machine were optimised using Bayesian optimisation across 15 compounds on Latin Hypercube sampled data. We show, that while the post-column neutralisation with n-butylamine boosts the performance on the timsTOF machine by 43%, the same setup reduces the signal intensity and S/N ratios on the QQQ, indicating that this approach is not generally suitable for all instrumental setups. Furthermore, we showcase initial profiling results of naïve mouse lung tissue, revealing a specific set of highly abundant oxylipins. Overall, our work sets the stage for the comprehensive profiling of oxylipin abundance in tissue, in order to advance our understanding of tissue-specific immunity.

POSTER 12

LINDOLIN ALKALOIDS SELECTIVELY SUPPRESS MPGES-1 EXPRESSION AND PRESERVE PRO-RESOLVING FUNCTIONS IN HUMAN MACROPHAGES

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Screening of a library of *Linderina pennispora*-derived alkaloids and semisynthetic analogues structurally related to the antiallergic drug tranilast in human macrophages identified lindolin A and its semisynthetic congener lindolin C as potent suppressors of prostaglandin E2 (PGE2) formation, while largely sparing other lipid mediators. Rather than directly inhibiting microsomal PGE2 synthase-1 (mPGES-1) activity, lindolins and tranilast suppressed mPGES-1 gene expression during lipopolysaccharide (LPS)/interferon-gamma-induced polarization of human macrophages toward an M1 phenotype, thereby reducing mPGES-1 protein levels and PGE2 production. Lindolin activity was largely independent of mitogen-activated protein kinase (MAPK) signaling, as only minor changes on the phosphorylation of p38 MAPK, JNK, and ERK-1/2 were observed. In addition, macrophage surface marker analysis revealed no significant effects of lindolins or tranilast on M1/M2 polarization. Cytokine profile analysis in M1-macrophages showed that lindolins A and C significantly reduced IL-6 and TGF-beta while slightly increasing IL-10. Comparable effects were observed in monocytes, whereas M2-macrophages showed a trend toward increased TGF-beta production due to lindolin A. In contrast, tranilast did not significantly alter cytokine release in any cell type. Lipid mediator profiling of exotoxin-stimulated cells revealed that lindolin A preserved pro-resolving mediator formation in M2-macrophages, whereas tranilast reduced multiple prostanoids, 12/15-lipoxygenase products, and resolvin D5; these effects were stimulus-independent. In summary, lindolin A selectively suppresses inflammatory PGE2 formation by interfering with mPGES-1 expression while preserving pro-resolving lipid mediator pathways, suggesting a favorable immunomodulatory profile compared to tranilast.

POSTER 13

THE EFFECT OF LIPID MEDIATORS ON TUMOR PROGRESSION AND INTERACTION WITH MICROGLIA IN A 3D BRAIN TUMOR IN VITRO MODEL

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Bioactive lipids are a versatile group of molecules that take part in many essential cellular functions. In addition to physiological roles, bioactive lipids have been identified to play a role in different pathologies, for example as mediators in cancer biology. However, many details regarding lipid mediators in brain tumors are still unknown. In this project, the aim is to study lipid mediators relevant for glioblastoma and their role in mediating inflammatory response in the brain tumor microenvironment (TME). 2D cultures of human glioblastoma and microglia cell lines are used to characterize expression of cyclooxygenases (COX) and lipoxygenases (LOX) and secretion of prostaglandins and leukotrienes in vitro. To mimic the TME better, a 3D hydrogel-based in vitro coculture model is developed, combining the human microglia and glioblastoma cells. The goal is to use the established 3D model for testing whether specific lipid mediators, for instance prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), change inflammatory response of microglia, and if inhibition of cyclooxygenase and lipoxygenase pathways reduces proliferation and invasion of glioblastoma cells. We investigated glioblastoma invasion and microglia state in 3D model by immunofluorescence staining and cytokine release, revealing that the presence of microglia reduced glioblastoma spheroid invasion and increased the levels of some pro-tumorigenic factors in the culture environment. Only low expression levels of COX-1, COX-2, 5-LOX and 15-LOX were detected by qPCR and western blot in glioblastoma cells and microglia, in parallel with minimal secretion of PGE2 and LTB4 detected by ELISAs. Still, the treatment with COX and LOX inhibitors affected the growth of glioblastoma spheroids with or without microglia. Combining lipid mediator analysis with improved 3D glioblastoma models will be an important next step in the field. Gained knowledge on the promoting and inhibiting functions of lipid mediators in the brain tumor microenvironment could open possibilities for new cancer therapy targets, possibly leading also to reduced chemotherapy resistance and improved treatment effectiveness.

POSTER 14

INTEGRATING LIPID MEDIATOR PROFILING AND METABOLOMICS TO EXPLORE RESOLUTION PHARMACOLOGY IN THE SOUTH AFRICAN MEDICINAL PLANT

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Background: Traditional medicinal plants are complex chemical systems that remain largely unexplored within lipid mediator research. *Tetradenia riparia* (Hochst.) Codd is a South African medicinal plant traditionally used across Southern Africa to manage musculoskeletal pain, headaches, and inflammatory conditions. However, its ability to regulate lipid mediator networks and engage endogenous resolution pathways remains unknown. Objectives: To investigate the immunomodulatory and pro-resolving effects of *T. riparia* extracts and fractions in human macrophages using an integrated resolution pharmacology and metabolomics approach. Methods: Human peripheral blood mononuclear cell-derived macrophages (obtained from 7-day culture) were stimulated with lipopolysaccharide (LPS) and treated with T. riparia dichloromethane (DCM) extract and bioactive enriched fractions. Expression of inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8), signalling mediators (STAT3, JAK2, and PI3K), and resolution-associated determinants (ALOX12, ALOX15, GPR18, and GPR32) was quantified by qPCR. Macrophage functional responses were assessed using pHrodo™ Red-based efferocytosis assays. Targeted LC-MS/MS lipid mediator profiling quantified prostaglandins, leukotrienes, and specialized pro-resolving mediator pathway intermediates. In parallel, the chemical space of the DCM plant extract was characterised by LC-UV/HRMS and untargeted LC-MS/MS molecular networking. Results: *T. riparia* significantly suppressed pro-inflammatory cytokine expression and downregulated STAT3, JAK2, and PI3K signalling pathways. Concurrently, lipid mediator profiling revealed increased levels of 17-HDPA together with modulation of prostaglandin and leukotriene pathways. These changes were accompanied by upregulation of ALOX12, ALOX15, GPR18, and GPR32, supporting engagement of endogenous resolution programs. Notably, bioactivity-guided fractionation uncovered distinct functional activities. Although the parent DCM leaf extract displayed limited effects on macrophage efferocytosis, fractions FR-5 and FR-6 enhanced apoptotic cell clearance relative to untreated controls, highlighting the value of chemical deconvolution of complex botanical mixtures. Furthermore, LC-UV/HRMS-guided metabolomic analysis revealed extensive unexplored chemical diversity, with over 90% of detected molecular features lacking spectral matches in current natural product databases. *Conclusions*: *T. riparia* promotes coordinated regulation of inflammatory signalling, lipid mediator pathways, and macrophage efferocytic function. These findings demonstrate how integration of lipid mediator profiling, functional resolution assays, and metabolomics can uncover novel pro-resolving activities from African medicinal plants and support the development of multi-component therapeutic strategies for inflammatory diseases.

POSTER 15

CAPACITIES OF HUMAN MACROPHAGES AND LIPOXYGENASE-EXPRESSING HEK293 CELLS TO FORM FREE AND ESTERIFIED OXYLIPINS

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Oxylipins represent a class of signalling molecules that arise from the oxygenation of polyunsaturated fatty acids (PUFAs) and fulfil diverse functions in homeostasis or inflammatory conditions. Besides their paracrine action as soluble mediators, various oxylipin species can occur esterified in phospholipids, either via non-enzymatic or direct enzymatic oxidation of phospholipid-bound PUFAs or via the re-esterification of oxylipins formed from free PUFA substrates. The latter two mechanisms give rise to enzymatically oxidised phospholipids (eoxPLs), which are characterised by regio- and stereospecific oxygenation depending on their enzymatic origin. Among the enzymes crucially involved in eoxPL formation are lipoxygenases (LOXs) and of the six human isoforms direct phospholipid oxygenation has been reported for 15-LOX-1 (ALOX15) and 15-LOX-2 (ALOX15B). Several immune cells show differential expression of LOX isoforms and context-dependent immunomodulatory effects have been suggested for eoxPLs derived from this enzyme family, which most prominently include phospholipids carrying mono-hydroxylated (mono-OH) PUFAs. However, a comprehensive understanding of LOX isoform-dependent eoxPL formation under different (patho-)physiological conditions is lacking. Here, we reveal differences in the capacity of selected LOX isoforms to form esterified oxylipins under different stimulatory conditions using monocyte-derived macrophages (MDMs) and transfected HEK293 cells expressing 5-LOX, 15-LOX-1 or 15-LOX-2. Through side-by-side comparison of free oxylipins and total oxylipins obtained via saponification, we found higher levels of esterified mono-OH oxylipins under resting conditions. This was especially pronounced for MDMs and transfected HEK293 cells with strong expression of 15-LOX-2 rather than 15-LOX-1. On the other hand, stimuli like Staphylococcus aureus-derived exotoxins or the natural product acetyl-11-keto-β-boswellic acid (AKBA) induced strong formation of 15-LOX-derived mono-OH products but mostly diminished differences between free and total oxylipin amounts. This suggests efficient liberation and/or negligible re-esterification of mono-OH 15-LOX products under such conditions. Taken together, our data contributes to a better understanding of LOX product formation and specifically suggests context-dependent activity of 15-LOXs, contributing to either the pool of eoxPLs or free oxylipins.

POSTER 16

GLUCOCORTICOID- AND NSAID-LIKE EFFECTS OF PANAX GINSENG COMPOUNDS ON THE LIPID MEDIATOR NETWORK

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Background: Glucocorticoids (GCs) and non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammation, primarily by targeting cyclooxygenase (COX)-mediated prostaglandin formation. GCs suppress COX-2 expression, whereas NSAIDs inhibit COX catalytic activity. In addition, GCs regulate 15-lipoxygenase (15-LOX) isoform expression and thereby influence the biosynthesis of specialized pro-resolving mediators (SPM) [1]. Ginsenosides from Panax ginseng exhibit immunomodulatory properties, but their impact on lipid mediator networks is not fully understood. Here, we investigated whether ginsenosides differentially regulate prostaglandin, leukotriene, and SPM biosynthesis. Methods: LOX pathway regulation was analyzed in primary human innate immune cells treated with ginsenosides. Lipid mediator profiles were assessed by targeted metabololipidomics (UHPLC-MS/MS) and correlated with LOX expression at the mRNA and protein levels in polarized M1-/M2-like monocyte-derived macrophages. Pharmacological approaches were used to evaluate the involvement of glucocorticoid receptor signaling. Results: Depending on the glycosylation pattern and stereochemistry, ginsenosides combined NSAID-like inhibition of COX-derived prostaglandin and 5-LOX-derived leukotriene formation with GC-like inverse 15-LOX regulation (robustly blocking IL-4-induced 15-LOX-1 while moderately inducing 15-LOX-2) [2]. Notably, ginsenosides acted independently of the glucocorticoid receptor and yielded additive effects with low-dose GC co-administration. COX-2 expression was not affected. Overall, LOX inhibition prevailed, reflected by reduced formation of 15-LOX-dependent SPMs as well as reduced 5-LOX-dependent leukotriene formation. *Conclusions*: These findings demonstrate the potential of ginsenosides to differentially modulate LOX and COX pathways at the levels of enzymatic activity and gene expression, thereby shaping early pro-inflammatory and late pro-resolving lipid mediator networks. Decoupling functional activity from expression enables selective modulation of inflammatory responses.

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POSTER 17

DIFFERENTIAL REGULATION OF CIRCULATING AND BILIARY N-ACYL TAURINES DURING LIVER INJURY

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Background: N-acyl taurines (NAT) are taurine-conjugated fatty acids present in plasma and bile that rapidly accumulate upon inhibition of the hydrolytic enzyme fatty acid amide hydrolase (FAAH), a feature shared by many potent signaling molecules. In mice, unsaturated NATs have shown beneficial effects on metabolic health. Hepatic NAT synthesis and transport overlap with those of conjugated bile acids, requiring fatty acid transport protein 5 (FATP5) and bile acid-CoA:amino acid N-acyltransferase (BAAT) for hepatic synthesis. Previously, we observed elevated plasma NAT levels in humans with metabolic dysfunction-associated steatotic liver disease (MASLD) and following acute overfeeding. However, whether NAT regulation extends beyond hepatic lipid accumulation, and the mechanisms driving these changes, remain unclear. Methods: Plasma NAT levels were analyzed in humans with all-cause compensated liver cirrhosis, acute decompensation of cirrhosis, and acute lipopolysaccharide (LPS)-induced inflammation. Transgenic mouse models with impaired NAT hydrolysis or hepatic synthesis were treated with LPS to investigate contributions to plasma and bile NAT composition. Traceable NAT species were used to evaluate plasma clearance and biliary excretion in LPS-treated mice. Both in vitro and in vivo models were employed to investigate whether NATs modulate LPS-induced hepatic inflammation. Results: Plasma NATs were elevated during acute and chronic liver injury in humans and mice, with mice also exhibiting increased biliary NAT content. Although hepatic expression of FAAH and BAAT was altered following LPS exposure, plasma NAT elevations occurred independent of either enzyme. In contrast, LPS-induced increase in biliary NATs depended on BAAT-mediated synthesis. Tracing experiments suggested that LPS partially impaired plasma clearance potentially related to downregulation of hepatic NAT transporters. In vitro, NAT pretreatment attenuated LPS-induced expression of pro-inflammatory cytokines, whereas single-dose NAT administration in vivo neither ameliorated nor exacerbated the LPS response. Conclusion: NAT levels increase in acute and chronic liver injury without excessive lipid accumulation. Biliary NATs are primarily governed by hepatic synthesis, whereas plasma NAT elevations cannot be explained by altered hydrolysis, hepatic synthesis, or clearance alone, suggesting combined mechanisms or contributions from extrahepatic sources. Although acute dosing with NATs does not appear to influence injury severity in vivo, NATs may exert anti-inflammatory effects at the hepatocyte level.

POSTER 18

FORMULATION OF LIPID NANOCAPSULES FOR THE DELIVERY OF SPM

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The resolution of inflammation is an active and tightly regulated process orchestrated by several mediators, among which the specialised pro-resolving mediators (SPMs) play a central role. Increasing evidence suggests that chronic inflammation may arise from a failure of the endogenous mechanisms that normally terminate inflammation and restore tissue homeostasis. In the context of rheumatoid arthritis (RA), growing evidence indicates that the resolution phase of inflammation is impaired. While current treatments mainly aim to suppress inflammation using disease-modifying anti-rheumatic drugs (DMARDs), emerging strategies seek to actively promote the resolution of inflammation. This work aims to develop and characterise lipid nanocapsules (LNCs) to enhance the delivery and efficacy of pro-resolving lipid mediators. Indeed, the therapeutic application of several SPMs remains limited due to poor stability and bioavailability; therefore, their encapsulation represents a promising strategy to improve their delivery and possibly targeting to inflamed tissues. In this case, we aim to encapsulate both a lipid and a peptide. Indeed, besides lipid mediators, proteins such as Annexin A1 also exert pro-resolutive effects. This protein has also been described to decrease inflammatory pain. Maresin 2, a docosahexaenoic acid-derived SPM produced by macrophages, was encapsulated within LNCs using a modified phase inversion process. The composition of the LNCs includes DOTAP, a cationic surfactant which imparts a positive surface charge to the LNCs, enabling coulombic interactions with a hyaluronic acid coating functionalised with a pro-resolutive peptide derived from Annexin A1. Physicochemical characterisation of the LNCs was performed using dynamic light scattering (DLS) to determine particle size and surface charge, nuclear magnetic resonance (NMR) to confirm structure and purity, and HPLC-MS/MS to determine the Maresin 2 encapsulation efficiency as well as HPLC-UV to quantify the loading of the peptide derived from Annexin A1. Preliminary results demonstrate promising outcomes, with high encapsulation efficiency and suitable physicochemical properties, compatible with the administration of therapeutically relevant doses in small injection volumes for in vivo evaluation in RA mouse models. Altogether, these findings support the feasibility of LNC-based delivery systems as an innovative strategy to promote inflammation resolution in chronic inflammatory diseases.

POSTER 19

OXYLIPIN PROFILE TRAJECTORIES IN CANINE PREGNANCY: PLASMA AND URINARY SIGNATURES FOR DIAGNOSIS

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Identification of reliable, non-invasive biomarkers for early pregnancy detection in dogs is essential for sustaining optimal fertility and reproductive health. Gestation induces maternal immune adaptations that support fetal development, reflected in changes in oxylipin concentrations tied to oxidative stress and inflammatory responses.1 Oxylipins serve as key signaling lipids that harmonize immune and inflammatory processes during both normal and pathological pregnancies. These bioactive compounds, derived from polyunsaturated fatty acids (PUFAs) through enzymatic and non-enzymatic mechanisms, were the focus of this investigation. A new targeted panel comprising 90 oxylipins and 49 deuterium-labeled internal standards was developed for the diagnosis of pregnancy-related conditions. The analytical method, which utilized solid-phase extraction (SPE) and metal-organic framework microextraction (dSPE) followed by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-MS/MS) with a biphenyl LC column (2.1 x 150 mm, 2.6 µm), was systematically optimized and validated using design-of-experiments methodologies. Oxylipin concentrations were quantified in matched canine urine and plasma samples (Nplasma = 100, Nurine = 101, 24 breeds - from bitches during different stages of the reproductive cycle; urinary creatinine standardized to 0.25 mg mL-1) to facilitate gestational assessment. The assay encompassed cyclooxygenase, lipoxygenase, and cytochrome P450 pathways, enabling the detection of prostaglandins, isoprostanes, thromboxanes, leukotrienes, octadecanoids, eicosanoids, and hydroxyeicosatetraenoic acid derivatives. Of the 90 oxylipins analyzed, approximately 60 were detected above the limit of detection in at least 60% of samples and were included in statistical analyses. Among these, 8 plasma oxylipins and 12 urinary oxylipins demonstrated significant differences in abundance ($p < 0.05$) between pregnant ($n = 43$) and non-pregnant ($n = 17$) females, with pregnancy status confirmed by ultrasonography. Additionally, the concentrations of 11 plasma oxylipins and 29 urinary oxylipins differed significantly among the pre- ($n = 20$), during- ($n = 10$), and post-ovulation ($n = 12$) stages. These findings indicate that distinct oxylipin profiles associated with pregnancy status are detectable in both urine and plasma, supporting their potential as diagnostic markers for canine gestation.

POSTER 20

UNMASKING A MOLECULAR DECEPTION: cPLA2 INHIBITORS EMERGE AS POTENT LTA4H SUPPRESSORS

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Eicosanoids derived from arachidonic acid (AA) are key regulators of inflammation, modulating processes such as cell migration, proliferation, phagocytosis, and cytokine production. Upon stimulation, AA is released from membrane phospholipids by cytosolic phospholipase A2 (cPLA2) and subsequently metabolized via lipoxygenase (LOX), cyclooxygenase (COX), and cytochrome P450 (CYP) pathways. Among these metabolites, leukotriene B4 (LTB4), sequentially generated by 5-LOX and leukotriene A4 hydrolase (LTA4H), is a potent chemoattractant driving immune cell recruitment to sites of inflammation. We identified two commercially available compounds, CAY10641 and CAY10650, previously described as cPLA2 inhibitor (CAY10650) and its inactive phase-1 metabolite (CAY10641), as appearing to be more potent inhibitors of LTA4H. Both compounds selectively suppressed LTB4 formation across cellular and cell-free assay systems. Comparable to the reference LTA4H inhibitor LYS006 (IC50: 186 nM), they effectively reduced LTB4 biosynthesis (CAY10641: IC50: 37 nM, CAY10650: IC50: 36 nM) in A23187-stimulated neutrophils and redirected product formation from LTA4 toward non enzymatically generated e(t)LTB4 and lipoxins. The compounds exhibited approximately fivefold higher potency toward the epoxide hydrolase activity than toward the aminopeptidase function of the bifunctional LTA4H, without affecting soluble epoxide hydrolase. Docking studies and coincubation experiments with 5LOX activating protein (FLAP) inhibitors indicated the involvement of FLAP and identified e(t)LTB4 as a potential precursor of as yet unidentified oxylipins. Taken together, these findings indicate that CAY10641 and CAY10650 selectively inhibit LTB4 biosynthesis via direct interference with LTA4H and may uncover novel FLAP dependent oxylipin pathways.

POSTER 21

DECODING THE MACROPHAGE RESPONSE TO THE SECRETOME OF STAPHYLOCOCCUS AUREUS: LIPID MEDIATOR BIOSYNTHESIS AND PROTEOMIC LANDSCAPE

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Lipid mediators (LMs), a class of oxylipins, are key regulators of inflammatory processes. Pro-inflammatory stimuli trigger the liberation of polyunsaturated fatty acids, which serve as precursors for LM biosynthesis via cyclooxygenases, lipoxygenases, and cytochrome P450 enzymes. In this context, the oxylipin profile is influenced by various pathogenic virulence factors and differs between cell types. While the amphipathic alpha-helical phenol-soluble modulins (PSM) derived from *Staphylococcus aureus* (*S. aureus*) activate the 5-LOX pathway in neutrophils to form leukotrienes, the pore-forming toxin alpha-hemolysin of S. aureus stimulates the 15-LOX pathway in M2 macrophages. Elucidating the interaction between Staphylococcus aureus and macrophages in this context is essential, given the role of S. aureus in severe clinical infections worldwide. This study examined the induction of oxylipin biosynthesis by S. aureus exoproteomes (LS1, HG001, USA300, BOF782N, and D29) in M1-like macrophages. Bacterial secretomes were quantified and sublytic stimulation conditions were optimized by LDH and MTT assays. Subsequently, oxylipin profiles were obtained using UHPLC-MS/MS. Proteomic analysis following stimulation and pharmacological modulation revealed selective pathway-specific alterations in lipid metabolism and proteins involved in inflammation. For this purpose, macrophage oxylipin profiles were modulated using MK-886 (FLAP inhibitor), CAY10502 (cPLA2 inhibitor), and ibuprofen (COX inhibitor). Among the S. aureus strains compared, HG001 was identified as the strongest inducer of LM biosynthesis in M1-like macrophages. The HG001 exoproteome significantly modulated the macrophage proteome, altering abundances of 1617 out of 6336 detected human proteins. Gene Set Enrichment Analysis (GSEA) indicated a metabolic reprogramming of macrophages that supports antimicrobial activity against *S. aureus*. Notably, restricting the availability of polyunsaturated fatty acids through inhibition of cytosolic phospholipase A2 and the subsequent suppression of oxylipin biosynthesis resulted in decreased levels of several proteins associated with lipid metabolism and inflammation. Our findings establish *S. aureus* (HG001) exoproteomes as a potent inducer of macrophage oxylipin biosynthesis and proteomic remodelling, thereby promoting sustained antimicrobial defense. Furthermore, they highlight pathway-selective LM modulation as a promising anti-inflammatory strategy.

POSTER 22

EXPLORING THE ROLE OF 7ALPHA,25-DIHYDROXYCHOLESTEROL–GPR183 AXIS IN OBESITY-ASSOCIATED ADIPOSE TISSUE INFLAMMATION.

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Oxysterols, oxidized derivatives of cholesterol, are increasingly recognized as bioactive lipid mediators involved in the regulation of metabolic and immune processes. While their role as intermediates in bile acid synthesis is well established, emerging evidence highlights their contribution to the pathophysiology of obesity. Obesity is characterized by systemic low-grade inflammation, predominantly localized within adipose tissue. During disease progression, adipose tissue undergoes substantial immune cell infiltration, particularly by monocytes and B lymphocytes, thereby contributing to obesity-associated metabolic dysfunction. In this context, we investigated the potential involvement of the 7αlpha,25-dihydroxycholesterol (7alpha,25-diOHC)–GPR183 axis. This pathway has been extensively implicated in immune cell recruitment across various tissues, including the colon and lungs. In addition, 7α,25-diOHC has been reported to exert anti-lipogenic effects in hepatocytes. However, despite these observations, the role of this axis in the pathophysiology of obesity remains largely unexplored. Using peripheral blood mononuclear cells (PBMCs) isolated from high-fat diet-induced obese mice, we assessed the involvement of GPR183 in immune cell migration towards adipose tissue conditioned media. Pharmacological antagonism of GPR183 reduced cell migration, suggesting that this axis could contribute to immune cell recruitment in obesity. We further evaluated the effects of 7α,25-diOHC on adipose tissue inflammation using explants from obese mice. Treatment with 7α,25-diOHC led to a marked decrease in pro-inflammatory cytokine secretion in both subcutaneous and visceral adipose tissue depots. Altogether, our findings suggest that the 7α,25-diOHC–GPR183 axis could be involved in immune cell migration and inflammation in adipose tissue associated with obesity. Targeting this pathway may represent a promising strategy to modulate obesity-associated inflammation and metabolic dysfunction.

POSTER 23

CERAMIDE LEVELS ELUCIDATED IN ASTROCYTE REACTIVITY DURING ALZHEIMER'S DISEASE CLEAR - AD

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Alzheimer's disease (AD) is a fatal neurodegenerative disease with no effective treatment available. Astrocytes are crucial for maintaining brain homeostasis and their dysfunction can propagate AD pathogenesis. Accumulating evidence suggests that reactive astrogliosis occurs early during AD progression and an important role is emerging for altered lipid production and signalling, specifically by sphingolipids, during AD. Ceramides are considered key molecules in the complex sphingolipid biology. They can either contribute to inflammation and cell death or promote pro-survival signalling, depending on their chain-length. Recent studies have indicated that specifically reactive astrocytes are important producers of pro-apoptotic ceramides in the context of AD1, 2. However, the regulation of distinct ceramide species and their biosynthetic enzymes in astrocytes during neuroinflammation remains poorly understood. Here, we focus on the sphingolipid profile of human induced pluripotent stem cell (iPSC)-derived astrocytes, to elucidate the mechanism driving the shift towards more pro-apoptotic ceramides during neuroinflammation. This study will provide fundamental knowledge to better understand disease aetiology and assess whether restoration of the sphingolipid balance could serve as a potential therapeutic approach for AD.

POSTER 24

ELEVATION OF 12,13-DIHOME AND 9,10-DIHOME IN SEPSIS IS ACCOMPANIED BY UPREGULATION OF EPHX2, CYP2J2 AND CYP4F12

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12,13-DiHOME and 9,10-DiHOME are linoleic acid-derived oxylipins formed by the cytochrome P450 epoxygenase/epoxide hydrolase pathway, and may be implicated in sepsis-associated lung injury. Using the rat fecal peritonitis model, we confirmed that both 12,13-DiHOME and 9,10-DiHOME were significantly increased at 6 h and 24 h following sepsis induction and returned toward baseline by 48 h. Plasma 12,13-DiHOME concentration was highly correlated with pulmonary neutrophil activation as measured by MPO immunoreactivity. To assess whether genes related to this pathway are also altered in human sepsis, we analyzed 142 whole-blood transcriptomes from two independent studies, which included healthy controls, early infection, sepsis, septic shock and follow-up groups. We used a 60-gene panel covering cytochrome P450, epoxide hydrolase, cyclooxygenase, lipoxygenase and related pathways linked to oxylipin metabolism. Differential expression was assessed in R using DESeq2. Of the 60 genes examined, 45 were differentially expressed in at least one comparison, of which 15 genes were upregulated, 29 downregulated and 1 both up and downregulated across different comparisons. EPHX2 was the only gene significantly upregulated in all six comparisons (log2FC 1.32-2.41; adjusted p = 9.12e-12 to 5.63e-4), making it the most consistent upregulated hit in the panel. Because EPHX2 encodes soluble epoxide hydrolase, which converts EpOMEs to DiHOMEs, this finding supports the DiHOME-related changes observed in our rat model. CYP2J2 and CYP4F12, two cytochrome P450 enzymes linked to upstream EpOME generation, were also upregulated in three acute comparisons each (CYP2J2 log2FC 0.81-2.31; CYP4F12 log2FC 0.67-1.25), supporting activation of the same pathway. We therefore quantified protein levels of EPHX2, CYP2J2 and CYP4F12 in whole blood collected 24 h after fecal peritonitis induction or vehicle treatment in our rat model. In addition, upregulation of other enzymes involved in lipid mediator production, including ALOX15, PTGS2 and PTGDS, together with downregulation of enzymes involved in their inactivation, including HPGD and PTGR1, suggests that the observed increases in 12,13-DiHOME and 9,10-DiHOME in sepsis are accompanied by broader changes in inflammatory lipid pathway expression.

POSTER 25

IN SILICO INVESTIGATION OF HUMAN ALOX12 ALLOSTERIC REGULATION: COMPETITIVE CATALYTIC PATHWAYS OF 14S-HPDHA

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Maresin-1 is a specialized pro-resolving mediator derived from docosahexaenoic acid (DHA) that plays a key role in the resolution of inflammation. Its biosynthesis involves the enzymatic conversion of DHA into 14S-hydroperoxy-DHA (14S-HpDHA), followed by an epoxidation step leading to the key intermediate 13S,14S-epoxy-DHA. However, experimental studies have shown that 14S-HpDHA can also undergo a competing double oxygenation reaction to yield 7S,14S-diHDHA, and that the balance between these pathways may be regulated by allosteric effects, potentially involving the substrate itself as a modulator (1). In this work, we present a computational investigation of the molecular basis of this proposed allosteric regulation in human lipoxygenase 12 (hALOX12). Starting from a recently reported cryo-EM structure (8GHC, monomer A), we explored different substrate binding modes (head-first and tail-first) and investigated the presence of potential allosteric sites using molecular docking combined with allosteric site prediction methods. Extensive molecular dynamics simulations were performed to characterize substrate positioning and conformational dynamics of binary enzyme–substrate complexes, as well as ternary complexes including the allosteric effector. To evaluate the impact of allosteric binding on reactivity, QM/MM calculations were performed to explore the epoxidation mechanism, while oxygen access pathways were analysed to assess the feasibility of the competing oxygenation reaction. In addition, MM-PBSA calculations were used to estimate the effect of allosteric ligand binding on substrate affinity. Our results suggest that allosteric ligand binding modulates substrate positioning and destabilizes catalytically competent conformations required for epoxidation. Notably, the effect depends on the substrate binding mode: in the tail-first configuration, both epoxidation and oxygenation are disfavoured, whereas in the head-first binding mode, oxygenation is favoured over epoxidation, in agreement with the experimental observations. Overall, this study provides a molecular-level framework for understanding the experimentally observed shift in product distribution and highlights the role of allosteric regulation in controlling lipoxygenase reactivity, offering new insights into maresin biosynthesis and its modulation. (

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POSTER 26

EFFECTS OF OMEGA-3 FATTY ACIDS AND MARESIN-1 ON HUMAN CORONARY CONTRACTIONS INDUCED BY ELECTRICAL FIELD STIMULATION

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Introduction: Coronary artery disease (CAD) is characterized by chronic inflammation and increased production of neurotransmitters and pro-inflammatory lipid mediators, such as prostaglandin E2 (PGE2), responsible for vasoconstriction. Omega-3 are metabolized by lipoxygenases (5-LOX, 12-LOX, 15-LOX) into specialized pro-resolving mediators (SPM), such as maresins which are DHA metabolite, to resolve inflammation. Although omega-3 beneficial effects in patients with CAD have been described in clinical trials (1), and omega-3/SPM production by the human vagus nerve has been documented (2), their impact on the cardiac neuronal system remains unexplored. This study aimed to investigate the role of omega-3 and SPM in modulating (endogenous/exogenous) neurotransmitter-induced responses in human coronary arteries (HCA). We also explored the expression of enzymes involved in SPM synthesis. Methods: HCA were isolated from human hearts (n=6) post-transplantation at Bichat Hospital and placed in an organ bath system. Electrical field stimulation (EFS) at varying voltages was used to induce neurotransmitter release, before and after 1h incubation with DHA or EPA (0.1 mM) or maresin-1 (100 nM). Pharmacological treatments [tetrodotoxin (TTX, 10 microM), atropine (1 microM), prazosin (1 microM), and methysergide (1 microM)] were used to confirm the involvement of neuronal, cholinergic, adrenergic, and serotonergic pathways, respectively. To differentiate omega-3 pre- and postsynaptic actions, dose-response curves were generated using exogenous neurotransmitters. Immunofluorescence (IF) staining was performed to detect/localize LOX enzymes. Vascular tone was analyzed using IOX software. EFS-Contractions are expressed as % of the response obtained with the highest voltage. Results: EFS induced voltage-dependent contractions in HCA, partially inhibited by tetrodotoxin, atropine, and methysergide confirming a neuronal component mediated by specific neurotransmitters. DHA (0.1 mM) reduced significantly contractions by 68% at 10V and 39% at 30V. Contractions induced by exogenous neurotransmitters were not affected by omega-3, suggesting a presynaptic mechanism. IF analysis confirmed 12-LOX overexpression in HCA from CAD patients, an enzyme mainly involved in maresin-1 synthesis. In addition, 1-hour incubation with maresin-1 reduced EFS-induced contractions. Conclusion: DHA and maresin1 reduced contractions induced by EFS, alongside the increased expression of 12-LOX in CAD patients versus control. This suggests a potential role of the DHA/maresin pathway in neuromodulation.

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POSTER 27

UMBRELLA SAMPLING MOLECULAR DYNAMICS CAPTURES THE DETERMINANTS OF MEMBRANE PERMEABILITY WITH HIGH ACCURACY

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The crossing of drugs over the blood-brain barrier (BBB) is one of the most critical features enabling regulated access to the CNS. Predicting BBB permeability of leads and druglike compounds via the logBB metric is thus important in any rational drug design endeavour, either targeting CNS pathologies or not. The central outcome of this study is the optimization of a computational approach affording high correlation between experimentally determined logBB values and those predicted through Molecular Dynamics (MD) simulations performed using Gromacs or OpenMM software. By rigorously implementing the inhomogeneous solubility-diffusion framework within our theoretical pipeline, permeability coefficients derived from free energy profiles obtained by biased MD simulations and the corresponding position-dependent diffusion calculations were directly mapped onto experimental measurements. The resulting correlation remained consistently high, with Pearson r values around 0.79, while model refinement reduced the prediction error (RMSE decreased from 0.78 to 0.67 and MAE from 0.69 to 0.61), accompanied by a substantial increase in the coefficient of determination (R^2 from 0.50 to 0.62). A high frequency of interactions involving the formation of halogen bonds was observed between chlorinated compounds such as alprazolam, nordazepam and chlorpromazine and the membrane components. This difference directly influenced the permeation energetics and prompted the introduction of dummy atoms to explicitly model sigma-hole electrostatics in MD simulations, addressing a key limitation of additive force fields. At the membrane level, clear differences emerged between the additive CHARMM36 and the polarizable Drude force fields. The area-per-lipid decreased from 90.55 ± 6.34 $Å^2$ to 73.18 ± 0.14 $Å^2$, indicating significantly tighter lipid packing in the polarizable model. Order parameters showed modest but significant variations, suggesting a redistribution of acyl chain ordering rather than a uniform structural change. Crucially, the robustness of the observed correlation was reinforced by a chemically diverse dataset, which includes compounds spanning a wide range of polarity, molecular size and charge state. Overall, these results demonstrate that when polarization effects and halogen-specific interactions are precisely modelled, MD can serve as a competent predictive framework for assessing BBB and membrane permeability in general, of candidate drugs.

POSTER 28

DIFFERENTIAL CONTRIBUTION OF LIPOXYGENASE ISOFORMS TO THE SEQUENTIAL CELLULAR BIOSYNTHESIS OF SPECIALIZED PRO-RESOLVING MEDIATORS – THE ROLE OF MULTICELLULAR INTERACTIONS

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Oxylipins, produced from polyunsaturated fatty acids (PUFA), play pivotal roles in both inflammation and resolution. Key players in the acute phase of inflammation are cyclooxygenase-derived prostaglandins and 5-lipoxygenase (LOX)-derived leukotrienes. In contrast, the specialized pro-resolving mediators (SPM) are proposed to contribute to the resolution of inflammation. The biosynthesis of the SPM members lipoxins and resolvins (RVs) supposedly relies on two sequential PUFA oxygenation steps. Thus, 5-LOX and 12-/15-lipoxygenating paralogues (e.g., 12-LOX, 15-LOX-1/-2) are involved. These oxygenation steps may occur within a single cell where both LOX isoforms are expressed or via transcellular metabolism between different cell types that complementary express these LOXs. In order to address the discrepancy between in vivo-relevant multicellular events and monocellular in vitro models, we investigated (patho-)physiological relevant cell co-incubations, reflecting different stages of the immune response. In this context, co-incubations of human 5-LOX-positive polymorphonuclear leukocytes (PMNLs) or monocytes with 12-LOX-positive platelets or with 15-LOX-1-rich M2a or 15-LOX-2-rich M2Dex macrophages and respective monocellular incubations, were selected. Transfected HEK293 cells with single LOX isoform overexpression served as a supporting model. Comprehensive metabololipidomics using UPLC-MS/MS was employed to generate oxylipin profiles. While the interplay between 5-LOX and 12-LOX in lipoxin formation during PMNL-platelet and monocyte-platelet co-incubations was confirmed, a cooperation between 5-LOX and 15-LOX-2 yielding strong RvD5 and RvE4 formation was uncovered. In contrast, monocultured 15-LOX-1-rich M2a macrophages produced substantial RvD5, whereas co-incubation of M2a with 5-LOX-rich PMNLs impaired RvD5 production. Moreover, we demonstrate that 15-LOX-1 preferentially uses arachidonic acid and eicosapentaenoic acid as substrates. Conversely, 15-LOX-2 demonstrates a preference for docosahexaenoic acid and 5- or 7-monohydroxylated precursors for SPM formation. Together, given the interaction between different LOX-positive cells, the 5-LO/15-LOX-2 axis appears to exhibit stronger synergistic effects in the production of selected SPMs than the 5-LOX/15-LOX-1 axis.

POSTER 29

HIGH-RESOLUTION NANO-DESI MASS SPECTROMETRY IMAGING OF LIPID MEDIATOR DISTRIBUTIONS IN THE BRAIN

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Eicosanoids are essential signaling molecules in the brain that regulate neurovascular coupling, inflammation and neuronal signaling. They are also associated with pathological processes within the brain, such as stroke, traumatic brain injury (TBI) and neurodegenerative diseases, where they regulate inflammatory processes and injury responses. However, because eicosanoids are often produced in a spatially and temporally restricted patterns, their detection in bulk tissue samples can be challenging, due to signal dilution and matrix effects. In addition, conventional sample preparation methods frequently result in loss of important spatial and anatomical information. Mass spectrometry imaging (MSI) is a powerful technique for visualizing spatially resolvent metabolic changes across tissue sections. Nano-desorption electrospray ionization (nano-DESI) is an ambient MSI technique that uses a capillary liquid bridge to locally extract analytes from the tissue surface, followed by electrospray ionization mass spectrometry analysis. Nano-DESI is particularly well suited for quantitative MSI through the inclusion of internal standards in the extraction solvent. Consequently, nano-DESI provides a powerful and complementary technique for mapping molecular distributions in tissues. Previous nano-DESI studies have demonstrated highly selective and sensitive imaging of lipid mediators, such as prostaglandins, with through the incorporation of silver ions into the extraction solvent [1-3]. However, these studies were limited to a spatial resolution corresponding to 10 x 30 micrometer pixels [3]. Here we demonstrate high resolution imaging of lipid mediators in mouse and human brain tissues using a modified pneumatically assisted nano-DESI setup coupled to a Q-Exactive Orbitrap mass spectrometer. Imaging was performed at a pixel size of 10 x 10 micrometers. We further demonstrate distinct distributions of eicosanoids in human cerebral cortex and mouse cerebellum, highlighting the potential for high-spatial resolution mapping of lipid mediators in brain tissue.

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POSTER 30

PAMPS AND DAMPS FAIL TO ELEVATE THE SECRETION OF COX-2 DERIVED OXYLIPINS IN LONG-LIVED NAKED MOLE RATS

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Naked mole rats display many exceptional and unique traits that contribute to their extraordinary longevity. These features include insensitivity to pain, high tolerance of hypoxia, resistance to cancer, and a low incidence of age-associated diseases. Since innate immunity is intricately linked to these processes, naked mole rats are an intriguing and understudied model of healthy aging. One of the most well-established innate immune responses is the rapid induction of cyclooxygenase (COX)-2 after exposure to pathogen- (PAMPs) or damage-associated molecular patterns (DAMPs). On one hand, COX-derived prostaglandins (PG), particularly PGE2, initiate the host's immune response with pro-inflammatory outcomes but at later stages promote inflammation resolution and restore homeostasis. Whether naked mole rats align with this immunological concept has not been investigated. Here we show that macrophages and certain immunogenic organs of naked mole rats fail to elevate COX-2-derived prostaglandin levels after exposure to PAMPs or DAMPs. Compiling organ specific oxylipin profiles from 15 different tissues/organs of healthy naked mole rats essentially aligned with those of other rodents. However, and to our surprise, we found no elevation of prostaglandin biosynthesis in dissociated tissue cells that were exposed to lipopolysaccharides (LPS), lipoteichoic acid (LTA) or heme. Along these lines, stimulation of whole blood from naked mole rats with the abovementioned PAMPs and DAMPs again yielded only moderate prostanoid levels. Finally, ex vivo cultivated bone marrow-derived macrophages from naked mole rats, which in other species possess high capacities to produce COX-2-derived prostanoids, exhibited no prostaglandin formation upon stimulation with PAMPs or DAMPs. These findings suggest conserved alterations in prostanoid biosynthesis in naked mole rats, which, given the relevance of prostanoids in pain sensation, cancer development and aging, could be at the core of features that contribute to their longevity. Future research on long-living species like the naked mole rat alongside studies on human centenarians may allow the identification of oxylipin-based response circuits, which potentially could be exploited to extend human life- and health span.

POSTER 31

PLASMA DHA AND OXYLIPIN PROFILES ARE ASSOCIATED WITH PHENOAGE ACCELERATION

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Biological aging has been associated with omega-3 polyunsaturated fatty acids (n-3 PUFAs); however, evidence specifically concerning docosahexaenoic acid (DHA) in relation to Phenotypic Age (PhenoAge) acceleration remains limited. Moreover, the role of PUFA-derived oxylipins in this context has not been thoroughly characterized. Therefore, this study investigated the associations of plasma DHA and PUFA-derived oxylipins with PhenoAge acceleration in an adult cohort. Samples from 156 individuals recruited at Brandenburg Medical School, University Hospital Ruppin-Brandenburg, Germany, were analyzed and categorized into quartiles of PhenoAge acceleration. Plasma and erythrocyte PUFAs were quantified by GC-FID, whereas oxylipins were quantified by HPLC-MS/MS. Associations with PhenoAge acceleration were assessed using non-parametric tests and multivariable robust regression, with Bonferroni and FDR correction applied. Plasma DHA concentrations declined progressively across increasing quartiles of PhenoAge acceleration, with a significant difference between the extreme quartiles. In multivariable analyses, higher plasma DHA was associated with lower PhenoAge acceleration, whereas the composite DHA+eicosapentaenoic acid (EPA) index showed a weaker inverse association. Comparable patterns were observed in erythrocytes. PUFA-derived oxylipins exhibited precursor-specific trends: n-3 PUFA-derived oxylipins were inversely associated with PhenoAge acceleration, whereas n-6 PUFA-derived oxylipins showed positive associations, with arachidonic acid-derived epoxyeicosatrienoic acids reaching statistical significance. Taken together, higher plasma DHA levels were associated with lower PhenoAge acceleration, with a stronger effect size compared to the composite DHA+EPA measure. Oxylipin profiles further indicated pathway-specific contributions of PUFA metabolism, highlighting distinct lipid metabolic signatures of biological aging. These findings suggest that DHA and PUFA-derived oxylipins may play a role in modulating biological aging processes, providing a basis for future mechanistic and interventional studies.

POSTER 32

BLOOD MULTIOMICS IDENTIFY MOLECULAR FEATURES OF DYSREGULATED LIPID METABOLISM AND MITOCHONDRIAL DYSFUNCTION ASSOCIATED WITH THE OUTCOME OF ACUTELY DECOMPENSATED CIRRHOSIS

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Abstract: The mechanisms driving the progression from acutely decompensated cirrhosis (ADC) to acute-on-chronic liver failure (ACLF), a condition associated with high mortality, remain poorly understood. Here, we integrated multi-omics and clinical data from 766 ADC patients to construct multiscale, multifactorial response networks associated with two major ADC outcomes: ACLF development and death. Of the 291 features included in the integrated analysis, 22 were associated with ACLF development and 16 with mortality. Features linked to imminent risk of ACLF and death formed a network connecting markers of mitochondrial dysfunction with the accumulation of the arachidonic acid-derived lipid mediator 20-hydroxyeicosatetraenoic acid (20-HETE). This network was experimentally validated in peripheral leukocytes, where 20-HETE was shown to induce mitochondrial oxidative stress and impair mitochondrial respiration via a GPR75-Akt signaling pathway. Finally, the network features served as early predictors of ADC outcomes, a finding that was independently validated in an external cohort of 580 ADC patients.

POSTER 33

SIMULTANEOUS CHARACTERIZATION OF QUEEN BEE-HEAD LIPIDS AND PHEROMONES USING 4D-LIPIDOMICS

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Introduction Bees are fundamental for maintaining biodiversity and ecosystem health, with their hives operating as highly organized social systems. The comprehensive analysis of lipids and pheromones in queen bee heads is critical for advancing our understanding of the biochemical foundations of social communication in honeybees. Queen pheromones act as indicators of reproductive status, shaping worker behavior and colony organization. Previous research has demonstrated that worker acceptance is more closely associated with age-dependent physiological changes than with direct reproductive metrics, underscoring the biological significance of this analytical approach [1]. However, the simultaneous characterization of lipids and pheromones from queen bee heads presents substantial analytical challenges due to the complexity and diversity of the molecular species involved. Bruker's novel timsMetabo platform addresses these challenges by combining the selectivity of trapped ion mobility spectrometry (TIMS) with high analytical sensitivity and broad mass range coverage, enabling precise and selective profiling of biologically and chemically complex samples. Here, we present an LC-TIMS-MS/MS approach developed to differentiate the pheromone and lipid profile of virgin and mated queen bee-heads. Methods Queen bee-head samples (virgin and mated) were homogenized and extracted using MTBE into lipid and metabolite fractions. These extracts were subsequently analyzed on the novel timsMetabo platform. Lipid profiling was performed using a Waters Acquity CSH C18 column with a total runtime of 18 min. Data acquisition was carried out based on the mobility range-enhancement (MoRE) acquisition mode in both positive and negative ionization modes. The acquired data was processed and analyzed in Bruker MetaboScape 2025b. Results The advanced analytical capabilities of the timsMetabo platform enabled comprehensive, rule-based annotation of diverse lipid classes, including phospholipids, sphingolipids, and neutral lipids. Furthermore, the implementation of the novel MoRE acquisition mode facilitated the simultaneous detection of pheromones by optimizing the transfer of low-mass metabolites alongside higher-mass lipids. The TIMS-enhanced 4D-Lipidomics approach allowed for a detailed biochemical characterization of the queen bee-head samples from virgin and mated populations, indicating that maturity-dependent physiological changes in queen bees are reflected in the lipidome.

[1] McAfee, A.; Magaña, A. A.; Foster, L. J.; Hoover, S. E. *iScience* 2024, 27 (10).

POSTER 34

COMPARATIVE FATTY ACID PROFILING OF MURINE RETINAL DEGENERATION MODELS HIGHLIGHTS DIVERGENT DISEASE PATHWAYS

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Although inherited retinal dystrophies share common features, such as inflammation and metabolic stress, distinct molecular mechanisms may underlie each disease model. Fatty acids are key modulators of inflammation and neuronal survival, and photoreceptors are particularly enriched in specific fatty acids that contribute to the membrane curvature required for disc formation and influence the functionality of proteins involved in phototransduction and synaptic transmission. We hypothesize that fatty acid alterations may be disease-specific and that their dysregulation may exacerbate neuronal damage. To explore this, we analysed the retinal fatty acid profile in two murine models of photoreceptor degeneration: one had a PRPH2 mutation that altered outer segment structure, and the other had a PDE6 mutation that disrupted phototransduction. Retinal fatty acids were extracted using Folch's method and transesterified to fatty acid methyl esters for their analysis. Fatty acids ranging from C8 to C28 were resolved by gas chromatography coupled to mass spectrometry and quantified. Also, transcriptomic analysis was performed using total RNA sequencing and enrichment of fatty acid-related biological processes. While some fatty acids were commonly altered in both models, the changes in others were specific, indicating different types of lipid remodelling depending on the pathways affected, rather than on net photoreceptor death. We demonstrated that the fatty acid profile is highly dynamic, depending not only on genotype but also on the animal's age. We found that some fatty acids, such as myristic acid in the PDE6 mutation model, were directly involved in disease onset. However, we also found fatty acids involved in pathways that could indirectly worsen the degenerative process, such as palmitoleic acid in mice with a PRPH2 mutation, a fatty acid that mitigates endoplasmic reticulum stress. Despite both models exhibited photoreceptor cell death, the fatty acid profile was disease-specific, suggesting different molecular mechanisms occurring during retinal degeneration. Data obtained from lipidomics may help us better understand the molecular mechanisms behind degeneration.

POSTER 35

TP RECEPTOR ANTAGONISM SUPERIOR TO BIOSYNTHESIS INHIBITION FOR BLOCKADE OF THE PROSTANOID COMPONENT IN IGE TRIGGERED HUMAN SMALL AIRWAY CONSTRICTION

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Introduction IgE-dependent mast cell activation induces prostanoid release, but the contribution of prostanoids to constriction of human small airways remains unclear. Aim and objective To define prostanoid-dependent mechanisms involved in anti-IgE-induced contraction of freshly isolated human small airways. Methods Human small airways less than 2 mm in diameter from 30 donors were studied in organ baths using receptor antagonists and enzyme inhibitors. Prostanoid release was quantified by enzyme immunoassay. Data are presented as mean +/- SEM. Results Anti-IgE at 5 microgram/ml caused marked contraction of human small airways, with an Emax of 89 +/- 8%. Combined antagonism of histamine H1 receptors with mepyramine and CysLT1 receptors with montelukast reduced the response to an Emax of 52 +/- 6%. The remaining contraction was abolished by inhibition of cyclooxygenase 1 with FR122047, thromboxane synthase with ozagrel, or TP receptor antagonism with SQ-29548. Replacing ozagrel with inhibition of hematopoietic prostaglandin D synthase using PPCA augmented contraction, with an Emax of 67 +/- 7%. Anti-IgE increased release of thromboxane B2, prostaglandin D2 and prostaglandin F2alpha to 38 +/- 22, 262 +/- 104 and 61 +/- 19 pg/mg, respectively. PPCA suppressed prostaglandin D2 to 12 +/- 3 pg/mg and increased thromboxane B2 to 122 +/- 32 pg/mg, whereas ozagrel suppressed thromboxane B2 to 8 +/- 3 pg/mg and increased prostaglandin D2 to 362 +/- 172 pg/mg. Release of prostaglandin F2alpha increased with combined ozagrel and PPCA to 134 +/- 13 pg/mg. Under H1 and CysLT1 blockade with ozagrel and PPCA, a residual contraction reemerged, with an Emax of 36 +/- 7%. This was reduced by prostaglandin F synthase inhibition with CRT0036521 to an Emax of 13 +/- 5%, similar to TP receptor antagonism. Conclusion Contractile prostanoids mediate IgE-induced narrowing of human small airways through TP receptors, activated by thromboxane A2, prostaglandin D2 and prostaglandin F2alpha. Selective inhibition of individual prostanoid pathways was associated with shunting between prostanoid products. Clinically, TP receptor antagonism may be preferable for suppressing the prostanoid component of mast cell-driven bronchoconstriction.

POSTER 36

BACTERIAL CHOLESTEROL-DEPENDENT CYTOLYSINS EVOKE FORMATION OF PRO-RESOLVING LIPID MEDIATORS VIA THE MAC-INHIBITORY PROTEIN CD59

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Cholesterol-dependent cytolysins (CDCs) are a class of pore-forming exotoxins and key virulence factors of several Gram-positive bacteria, including *Streptococcus pneumoniae* (pneumolysin) and *Streptococcus intermedius* (intermedilysin). These proteins bind membrane cholesterol to form large transmembrane pores, allowing the flux of ions and small molecules as well as the leakage of larger intracellular components, resulting in cell lysis and inflammation. While the general effects of CDCs, including activation of mitogen-activated protein kinase (MAPK) signaling, inflammasome priming, and cytokine release are well established, their impact on the formation of bioactive lipid mediators in innate immune cells has not been systematically addressed. Here, we demonstrate that CDCs elicit distinct lipid mediator profiles in various innate immune cells. In detail, pro-inflammatory M1-like monocyte-derived macrophages and neutrophils respond to CDCs with robust formation of cyclooxygenase (COX)-derived lipid mediators. In contrast, anti-inflammatory M2a-like monocyte-derived macrophages generate 15-lipoxygenase–derived specialized pro-resolving lipid mediators (SPMs), accompanied by rapid calcium influx and activation of p38 MAPK signaling. Unlike pneumolysin, intermedilysin does not initially bind membrane cholesterol. Instead, it first engages the human complement regulatory protein CD59, which then facilitates subsequent cholesterol-dependent membrane insertion and pore formation. We show that blocking CD59 on the cell surface reduces membrane damage, calcium influx, and SPM formation, indicating a key role for CD59-dependent membrane interactions in cytolysin-induced signaling and immune activation. These effects correlate with the differential expression of CD59 across innate immune cell subsets. Our findings demonstrate that CDC-induced membrane stress drives phenotype-specific lipid mediator profiles in innate immune cells, promoting pro-inflammatory COX responses in M1-like cells while inducing pro-resolving SPM pathways in M2a-like macrophages, thus affecting both initiation and resolution phases of inflammation. Moreover, the crucial role of CD59 offers new opportunities to utilize (small) molecules that target CD59 for modulating lipid mediator networks in innate immune cells.

POSTER 37

PROSTANOIDS RECEPTORS MODULATOR IN RAT HEMORRHAGIC CYSTITIS. CAN SELEXIPAG AND ALPROSTADIL REVERSE IT?

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Background and aim Acetylcholine (ACh) and adenosine triphosphate (ATP) are the major neurotransmitters involved in the bladder contraction. The current study aims to investigate the modulatory role of some prostanoids in cholinergic and purinergic pathways in normal status and hemorrhagic cystitis and their potential interaction with NO/cGMP pathway and to evaluate the role of selexipag and alprostadil in the management of this disease. Method Experiments were performed using male Wistar rats weighing (180-280 g) obtained from the animal house of Faculty of Pharmacy, Alexandria University. Rats were kept at room temperature with free access to chow (19% proteins, Elfagr Co., Egypt) and water. The laboratory animal care principles were strictly followed in all research experimental protocols and procedures as approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmacy, Alexandria University. (Approval Number: AU06.2020.1.12.1.67) Hemorrhagic cystitis was induced in rats by single injection of cyclophosphamide (300 mg/kg) and confirmed histopathologically. Organ bath experiments were implemented using isolated detrusor muscles. Bladder contraction was induced by electrical field stimulation (EFS) and by direct addition of ACh or ATP. Result and discussion Cystitis attenuated detrusor muscle contractility. Alprostadil (synthetic PGE_1) and selexipag (IP agonist) potentiated EFS and ACh-induced contractions in normal and inflamed bladder. The Potentiation of neurogenic-induced contractions was significantly higher in cystitis in case of selexipag, while potentiation of ACh-induced contractions was significantly higher in cystitis in case of selexipag and alprostadil. At higher concentrations, selexipag and alprostadil potentiated ATP-induced contractions only in normal bladder. RO1138452 (IP antagonist) inhibited neurogenic and ACh-induced contractions in normal bladder and in cystitis. The same was observed in case of SC51322 (EP_1 antagonist) but the decrease in ACh- induced contractions was not significant in normal bladder. The inhibitory effect of RO1138452 on Ach (10^{-4} M)-induced contractions in inflamed bladder was significantly less compared to normal bladder. Both antagonists inhibited ATP-induced contractions in normal rats. S18886 (TP antagonist) inhibited ATP-induced contractions in normal bladder. Conclusions EP1, IP and TP receptors seem to play an important role in the purinergic signaling physiology of detrusor muscle contractility in normal conditions. EP1 and IP receptors seem to have a role in neurogenic and ACh-induced contractility of detrusor muscle contractility also in cystitis. Both selexipag and alprostadil may be promising in the functional regeneration of detrusor muscle activity in hemorrhagic cystitis and merit further investigation.

POSTER 38

MARINE CALCAREOUS ALGAE AS ALTERNATIVE SOURCE OF LIPIDS: COMPARATIVE FATTY ACID AND OXYLIPIN PROFILING

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Microalgae are receiving increasing attention as a sustainable and natural source of food ingredients, because they contain valuable lipids, such as omega-3 and omega-6 fatty acids, as well as other bioactive compounds. Their potential as an environmentally friendly alternative to fish oils makes them especially relevant for nutrition and pharmaceutical applications. Fatty acids constitute the predominant lipid fraction in microalgae biomass, but they differ between species and are strongly influenced by the cultivation conditions. Furthermore, fatty acids can be enzymatically or autoxidatively converted into oxylipins, many of which remain unidentified and whose bioactivity is still largely unknown. This highlights the need for a comprehensive analytical workflow to characterize the fatty acid and oxylipin profiles that can be applied to different microalgae. This study characterized the fatty acid and oxylipin profiles of the coccolithophores *Emiliania huxleyi* and *Chrysotila carterae* using supercritical fluid chromatography - mass spectrometry (SFC-MS) and liquid chromatography - mass spectrometry (LC-MS), and compared them with those of the green microalga *Chlorella vulgaris*, which is already used as a food additive. The study aimed to investigate whether coccolithophores are suitable as an alternative source of fatty acids and whether their oxylipin profile differs systematically from that of the reference algae. The coccolithophores exhibited a fatty acid profile that was markedly distinct from that of *C. vulgaris*. In particular, significant differences between the algal species were observed in the levels of oleic acid and docosahexaenoic acid. For instance, *C. vulgaris* contains almost three times as much oleic acid as the coccolithophores, whilst the coccolithophores contain at least fifteen times as much docosahexaenoic acid. LC-MS-based oxylipin profiling likewise revealed clear interspecies differences. These results provide a basis for further comparative studies on algal lipid composition.

POSTER 39

PERORALLY APPLIED HYALURONAN ALTERS LIPID METABOLISM

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Introduction: The beneficial effects of hyaluronan (HA) as a dietary supplement have been shown in several studies; however, the exact biological mechanism remains unclear. Perorally applied hyaluronan (p.o.HA) is cleaved by the acidic pH of the stomach into fragments of approximately 50–600 kDa and is subsequently further degraded by the gut microbiome into unsaturated oligosaccharides (Šimek et al., 2023). Given that systemic absorption of orally administered HA is minimal (Šimek et al., 2023), we hypothesize that its effects likely occur through regulatory mechanisms in the intestine and by modulating metabolic processes. In line with this hypothesis, p.o.HA has been shown to alter lipid metabolism in UV-induced photoaged mice (Šimek et al., 2025). The aim of this study was to determine whether p.o. HA induces a shift in systemic lipid metabolism in healthy mice maintained on a standard diet. Material & Methods: Healthy mice C57BL/J fed a standard diet received drinking solutions for 14 days containing (1) HA, (2) pectin, or (3) water (control). Pectin was selected as a representative of a different polysaccharide for comparison. Untargeted lipidomic analyses of liver (HILIC) and plasma (RP-UHPLC-MS) were performed to compare these supplements and evaluate their effects on systemic lipid metabolism. Results: P.o. HA, but not pectin, alters lipid metabolism in both the liver and plasma, indicating the systemic effect. The concentrations of certain lysophosphatidylcholines (LPCs) and lysophosphatidic acid (LPAs) were significantly reduced. Various lipid mediators involved in organismal homeostasis, inflammation, and pain signaling were affected. BIOPAN implicated increased activity of membrane-bound O-acyltransferase domain enzymes and LPC acyltransferases. Discussion: P.o. HA affects shifts in lipid metabolism even at the systemic level; however, the mediator of these intestine-derived effects remains unknown. Furthermore, the reduced concentration of specific lipid mediators (e.g., LPA C18:1) following oral HA administration suggests that HA may also play a role in pain modulation; however, this requires further investigation.

1. Šimek M. et al., Molecular weight and gut microbiota determine the bioavailability of p.o.HA. *Carbohydr Polym*,2023, DOI:10.1016/j.carbpol.2023.120880. 2. Šimek M. et al., Alleviating UV-induced photoaging with p.o.HA: insights from a hairless mouse model. *Int J. Biol. Macromol.*, 2025. DOI:10.1016/j.ijbiomac.2025.146340.

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POSTER 40

SPECIALIZED PRO-RESOLVING MEDIATORS REGULATE IN VITRO HUMAN AIRWAY EPITHELIAL RESISTANCE TO ASPERGILLUS FUMIGATUS

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Cystic fibrosis (CF) is caused by mutations in the CFTR gene resulting in alterations in the immune functions of the airway epithelium, including reduced biosynthesis of the specialized pro-resolving lipid mediators (SPMs). *Aspergillus fumigatus* is the most isolated filamentous fungus in patients with CF. This study explores whether specialized pro-resolving lipid mediators (SPMs) regulate the barrier function of airway epithelium in vitro during colonization by *A. fumigatus*. We have shown that mature *A. fumigatus* hyphae disrupt tight junctions in primary cultures of nasal epithelium from CF and non-CF patients, as well as in a CF bronchial epithelial cell line. However, these cellular models mount an immune response to resist the fungus. We found that epithelial vulnerability is strongly dependent on its initial level of differentiation and integrity at the time of fungal inoculation. Indeed, while highly differentiated and non-CF epithelia exhibit a strong resistance against *A. fumigatus* and a significant reduction in fungal load and hyphal growth, less differentiated epithelia have a limited capacity to maintain their structure and contain fungal growth. Furthermore, we have demonstrated the role of several SPMs that stimulate the innate immune response of the airway epithelium by preventing tight junction disruption induced by *A. fumigatus* colonization and decreasing fungal galactomannan secretion. Moreover, SPMs increase hBD2 transcription in highly differentiated nasal primary cultures from non-CF subjects, but not in those from CF patients, and these lipid mediators reduced the production of pro-inflammatory cytokines induced by fungal colonization. Overall, our data suggest that the reduction of SPMs in patients with CF could contribute to the difficulty of eradicating *A. fumigatus* and this could open novel therapeutic perspectives to treat this disease.

POSTER 41

MOLECULAR ARCHITECTURE AND REGULATION OF THE INFLAMMATORY LEUKOTRIENE BIOSYNTHETIC PATHWAY

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Leukotrienes are highly potent bioactive lipid mediators derived from arachidonic acid that play central roles in inflammation, immune cell recruitment and host defense. Their biosynthesis is initiated by the leukotriene biosynthetic pathway, a tightly regulated enzymatic cascade centered on 5-lipoxygenase (5-LOX). The activity of 5-LOX is controlled by multiple cofactors and cellular signals, including calcium, ATP, membrane association and protein-protein interactions, which together regulate enzyme activation, localization and product formation. In addition to the native enzyme, a stabilized 5-LOX variant (Stable 5-LOX) has been widely used for biochemical and structural studies due to its enhanced conformational stability. However, this stabilization is accompanied by reduced catalytic activity, suggesting that intrinsic protein dynamics are important for efficient regulation and function. Understanding how regulatory signals act on both wild-type and stabilized forms of 5-LOX provides insight into the balance between structural stability and enzymatic responsiveness. Here, we investigate how different cofactors, including ATP and calcium, contribute to the regulation of human 5-LOX. Functional and mutational analyses indicate that ATP engages a regulatory site on the enzyme and modulates its activity in an allosteric manner. Comparative analyses between wild-type and Stable 5-LOX suggest that reduced activity of the stabilized variant arises from restricted conformational flexibility, which limits its ability to respond to regulatory inputs, such as ATP and calcium. Using an integrated approach combining structural biology, biochemical assays, site-directed mutagenesis and computational analyses of protein dynamics, we show that ATP binding alters long-range communication within the enzyme and influences its sensitivity to additional activation signals. These findings support a model in which 5-LOX activity is governed by cooperative regulation from multiple cofactors and highlight the importance of protein dynamics in leukotriene biosynthesis. More broadly, this work illustrates general principles by which lipid-metabolizing enzymes integrate metabolic and signaling cues to control inflammatory mediator production.

POSTER 42

Beyond prostaglandin E2: preclinical evaluation of prostaglandin E2 ethanolamide and prostaglandin E2 glycerol ester effects in acute lung injury

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Lung inflammation plays a crucial role in the pathology of many respiratory diseases and highlights the need for new therapeutic strategies. Prostaglandin glycerol esters (PG-G) and prostaglandin ethanolamides (PG-EA) are bioactive lipids derived from the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), respectively, through the action of cyclooxygenase-2 (COX-2) and specific prostaglandin synthases. We and others previously demonstrated that several PG-EAs and PG-Gs have anti-inflammatory effects in various models of inflammation. Here, we investigated whether these mediators could also modulate acute lung inflammation. This hypothesis was further supported by our previous findings showing that inhibition of alpha/beta-hydrolase domain-containing protein 6 (ABHD6) exerts anti-inflammatory effects and increases PGE_2 levels in lungs. In parallel, PGE_2 has been reported to have anti-inflammatory properties in models of lung inflammation. However, the effects of its derivatives, the glycerol ester (PGE_2-G) and ethanolamide (PGE2-EA), remain poorly studied. A comparative experimental approach was used to characterize the effects of PGE_2-G and PGE_2-EA in comparison with PGE_2. First, we evaluated their effects on LPS-induced activation of primary murine cells. Overall, PGE_2, PGE_2-G, and PGE_2-EA reduced LPS-induced cellular activation. We then investigated their effects in vivo using an experimental model of lung inflammation, focusing on three major hallmarks of the pathology: pulmonary cellular infiltration, local inflammatory response, and disruption of the alveolar-capillary barrier. Our results showed that some beneficial effects of PGE_2-G and PGE_2-EA were not fully recapitulated by PGE_2, suggesting mechanisms that are at least partially independent of PGE_2. Finally, we assessed the effects of these compounds on human PBMC and found that PGE_2, PGE_2-G, and PGE_2-EA decreased LPS-induced activation of these cells. Taken together, these findings suggest that PGE_2-G and PGE_2-EA may be promising bioactive lipids with potential therapeutic interest in lung inflammation.

POSTER 43

DEVELOPMENT OF A METHOD FOR THE COMBINED QUANTIFICATION OF N-ACYLPHOSPHATIDYLETHANOLAMINES AND N-ACYLETHANOLAMINES

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N-acylethanolamines (NAEs) are a family of lipid mediators that comprise the endocannabinoid N-arachidonoylethanolamine (AEA or anandamide) and the anti-inflammatory lipid N-palmitoylethanolamine (PEA). NAEs are involved in many physiological and pathological contexts. Several biochemical pathways are responsible for the production of NAEs with the key precursors being N-acylphosphatidylethanolamines (NAPEs). One of the key enzymes explored in this context is a NAPE-preferring phospholipase D (called NAPE-PLD) that hydrolyses NAPEs into the corresponding NAE and phosphatidic acid. Several studies, mainly based on NAPE-PLD knockout mice, have suggested the potential interest of interacting with this enzyme. Strikingly, only little is known about the biological effects of NAPEs and on how their levels are changed in physiopathological contexts. This is in part due to the limited number of described methods allowing to quantify NAPEs. As NAEs and NAPEs are biochemically linked, we aim at developing an LC-MS/MS method allowing us to quantify both NAPEs and NAEs in biological settings. First, we set up a chromatographic UPLC method using an HSS T3 column and optimized the parameters of the Waters® Xevo TQ-s mass spectrometer. Next, we optimized the extraction procedure and the solid phase extraction (SPE) pre-purification step. For the latter we aimed to remove the more lipophilic lipids, before eluting NAEs and NAPEs into the same fraction while leaving more polar lipids on the column. As there are no biological matrices devoid of the analytes of interest, we obtained the calibration curves by extracting and purifying the analytes from spiked surrogate matrices. The LLOD ranges between 50 to 250 femtomoles on column for the NAEs and 250 femtomoles on column for the NAPEs. QCs reached the intra- and inter-day accuracy, precision, and reproducibility criteria. Then we used this method to assess the ability of the NAPE-PLD inhibitor LEI-401 to modulate NAE and NAPE levels in vivo following i.p. administration to mice. The developed method should help improve our understanding of NAPEs and NAEs crosstalk.

POSTER 44

AN INVESTIGATION OF THE LIPID RECEPTOR (BLT1), NEUTROPHILS AND MACROPHAGES IN A ZEBRAFISH LARVAL MODEL OF INFLAMMATION, INJURY AND REGENERATION

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Neutrophils are a first line of defence, targeting pathogens and cellular debris by phagocytosis, NETosis, and influencing macrophage function. Macrophages phagocytose apoptotic neutrophils, promoting resolution of the immune/inflammatory responses. These processes of initiation, propagation and resolution of inflammation are modulated by key inflammatory receptors on leukocytes such as the powerful arachidonic acid metabolite lipid mediator leukotriene B4 (LTB4) receptor (BLT1), the cannabinoid receptors (CB1 and CB2), and the formylpeptide receptor (FPR1). We have investigated BLT1 and FPR1 in a larval zebrafish tailfin transection assay to model inflammation and compared the LTB4/BLT1 signalling response to N-Formylmethionine-leucyl-phenylalanine (fMLF)/FPR1 signalling response. Zebrafish larvae (up to 5 days post-fertilisation) are used for a number of reasons: optical transparency, genetic tractability and performing non-invasive, in vivo real-time imaging of fluorescently-labelled cells in an non-protected animal model. Transgenic zebrafish (Tg(mpx:GFP;mpeg1:mCherry)) with fluorescently tagged neutrophils and macrophages were used. Tailfin transection were conducted, and these leukocytes were imaged over 48 hours post-injury (hpi), showing an initial rise in neutrophil numbers within the first 8 hpi and a peak in macrophage numbers at 24 hpi, with both cell types returning to baseline by 48 hpi. Bathing the injured larvae in LTB4 increased both neutrophil and macrophage numbers, compared to control, in a concentration- and time-dependent manner. However, with the FPR1 agonist, fMLF, there was a significant rise exclusively in the neutrophil numbers, not macrophages. Incubation with antagonists of BLT1 (CP-105,696) and FPR1 (cyclosporin H) reduced the neutrophil and macrophage responses to injury, even when co-incubated with agonists. These antagonists also reduced the regeneration of the tailfin post-injury, in contrast to treatment with the agonists improving regeneration. These data show that targeting BLT1 or FPR1 affects inflammatory response/resolution, and tissue regeneration, indicating their potential as a therapeutic target. Interestingly, neutrophils and macrophages respond differently to LTB4 and fMLF, where we observed a surprising dissociation of neutrophil and macrophage accumulation into the injured tissue by the different agonist. Future work will involve using morpholino oligonucleotides to genetically and specifically knockdown some of these receptors of interest to examine their role more specifically.

POSTER 45

LONG-CHAIN ACYL-COA SYNTHETASE 4-MEDIATED LIPID REMODELING AND FERROPTOSIS DERIVE FIBROTIC PROGRESSION IN PULMONARY FIBROSIS

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Idiopathic pulmonary fibrosis (IPF) is a highly lethal disease with limited treatments, partly because the molecular mechanisms that link epithelial injury to progressive fibrosis are incompletely understood. Although dysregulated wound repair and oxidative stress are known to contribute to IPF pathogenesis, the upstream regulators controlling this process remain unclear. Our single cell RNA sequencing analysis revealed that expression levels of long-chain acyl-CoA synthetase (ACSL) 4 were elevated in fibroblasts of human IPF lungs. ACSL4 maintains highly unsaturated fatty acids-containing membrane phospholipid and regulates sensitivity to ferroptosis. In this study, we investigated the effect of ACSL4 gene deletion on bleomycin induced pulmonary fibrosis in mice. As the results, we found that wild type mice exhibited increased ACSL4 expression, marked lipid peroxidation, and progressive fibrosis by bleomycin administration, whereas ACSL4 deficient mice were protected from fibrotic remodeling and maintained higher oxygen saturation. Lipidomics demonstrated reduced the accumulation of lipid peroxides and arachidonic acid–derived metabolites in ACSL4 deficient lungs. Bone marrow chimeric experiments indicated that lung parenchymal cells, rather than immune cells, are critical compartments in which ACSL4 drives fibrotic progression. Furthermore, bleomycin-induced pulmonary fibrosis was significantly attenuated by pharmacological inhibition of ferroptosis during transitional and early progressive stages of fibrosis. These findings identify ACSL4 mediated lipid remodeling and ferroptosis of lung parenchymal cells as a key upstream driver of fibrotic progression. Targeting ACSL4 during tissue repair phase may represent a promising therapeutic strategy for pulmonary fibrosis.

POSTER 46

LOCALLY INCREASED 15-HETE IN MULTIPLE SCLEROSIS LESIONS AND ITS EFFECT ON HUMAN MICROGLIAL STATES

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A dysregulated bioactive lipid mediator (LM) profile may contribute to the chronic neuro-inflammatory nature of Multiple Sclerosis (MS). In particular, we have previously reported elevated plasma levels of the arachidonic acid (AA)-derivative 15-HETE, which correlated with reduced brain and increased lesion volumes in people with progressive MS (pwPMS). Yet, local 15-HETE levels within the MS brain and the putative effect of 15-HETE on neighboring central nervous system (CNS) cells remain elusive. In this study, we mapped the spatial distribution of 15-HETE in white matter MS brain tissue and non-neurological controls (NNCs) using mass spectrometry imaging (nano-DESI) analyses. We observed increased levels of 15-HETE in MS tissue, specifically in the core of the lesions. As 15-HETE can exist in two stereoisomers (15(*R*)-HETE and 15(*S*)-HETE) synthesized by distinct enzymes (e.g. COX-1/2 and ALOX15B respectively), we subsequently investigated the expression of these enzymes in human brain tissue from MS and controls. Here, we observed an increased expression of ALOX15B in tissue of pwPMS compared to HC, which was attributed to microglial subsets in single cell RNA sequencing datasets. The effect of ALOX15B-derived 15(S)-HETE was subsequently tested on IFNY/LPS-treated human iPSC-derived microglia (iMicroglia) by using bulk RNA sequencing. Here, differential gene expression analysis revealed a decrease of cytokine production (SOCS1/3/7, TGFB2), an increase of disease-associated microglial markers (Perilipin-2, FABP4, CD9) and lipid metabolism (PTGS2, ACSL1/4/5) upon 15(S)-HETE addition. Together, this project provides the first evidence of local 15-HETE biosynthesis within the CNS of pwPMS with a specific allocation to the core of MS lesions. With ALOX15B expression similarly being increased in WM lesions and microglia, we additionally reveal a disease-associated potential of ALOX15B-derived 15(*S*)-HETE on the microglial state in vitro.

POSTER 47

OXYLIPIN BIOSYNTHESIS BY FOAMY MACROPHAGES IN MULTIPLE SCLEROSIS LESIONS

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BACKGROUND + OBJECTIVE Multiple Sclerosis (MS) is a chronic, progressive disease of the central nervous system (CNS), characterized by immune-mediated neuroinflammation and neurodegeneration. The disease is defined by the formation of CNS lesions, some of which contain foamy myeloid cells with intracellular myelin debris, reflecting active demyelination and phagocytic activity. Recent evidence indicates that lesions containing foamy cells are enriched in oxidized metabolites derived from free fatty acids (oxylipins), suggesting that enhanced lipid metabolism within these cells may contribute to neuroinflammatory signaling and disease modulation. In this study, we hypothesize that foamy myeloid cells biosynthesize increased levels of oxylipins due to the enhanced availability of substrates derived from phagocytosed myelin, which is rich in polyunsaturated fatty acids-containing phospholipids. METHODS To test this hypothesis, human monocyte-derived macrophages were exposed to human myelin (100 µg/ml) for 72 hours to induce a foamy phenotype, after which the oxylipin profile was analyzed using liquid chromatography–mass spectrometry (LC-MS/MS). Moreover, expression of inflammatory and lipid-associated genes was determined by quantitative PCR. To assess whether oxylipin biosynthesis is mediated by enzymatic pathways, pharmacological inhibitors targeting key lipid mediator biosynthesizing enzymes were applied to the foamy myeloid cells. RESULTS In this study, we established an in vitro model to investigate foamy monocyte-derived macrophages, resulting in the development of a foamy phenotype characterized by intracellular lipid accumulation. Myelin-treated macrophages showed increased expression of genes associated with lipid droplet formation (PLIN2), cholesterol efflux (ABCA1), lysosomal degradation of phagocytosed material (CTSL), and inflammatory lipid mediator synthesis (PTGS2) as well as macrophage activation (SPP1). Preliminary LC–MS/MS analysis indicates that myelin uptake alters the oxylipin profile of foamy macrophages, suggesting that lipid loading may influence oxylipin production. Ongoing analyses aims to further characterize these changes and to determine whether oxylipin biosynthesis is mediated by specific enzymatic pathways. CONCLUSIONS Together, this study aims to define the oxylipin profile of foamy myeloid cells and determine whether myelin uptake drives oxylipin biosynthesis. Understanding how foamy macrophages contribute to lipid-mediated neuroinflammation may provide new insights into mechanisms underlying lesion progression in MS.

POSTER 48

FLAXSEED OIL CAKE AS A SOURCE OF BIOACTIVE COMPOUNDS: ANTI-INFLAMMATORY ACTIVITY AND EFFECTS ON LIPID MEDIATORS AND OXIDATIVE STRESS

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Flaxseed oil cake is an underutilized by-product of the oil industry with the potential to be valorised in a circular bioeconomy. In light of the growing interest in the sustainable utilisation of agricultural residues and the search for bioactive compounds with health-promoting properties, the anti-inflammatory potential of flaxseed oil cake-derived extracts and compounds was investigated. The present study focuses on the comprehensive characterisation of extracts obtained from golden and brown flaxseed oil cake. To capture the influence of extraction strategy and solvent polarity, samples were prepared using Soxhlet extraction, ultrasonic bath extraction, and bead-beater extraction with different extraction agents. The biological activity of the extracts was assessed by examining the inhibition of the biosynthesis of pro-inflammatory leukotriene B4 (LTB4), using both cell-free systems - consisting of isolated 5-lipoxygenase (5-LOX) and homogenates of polymorphonuclear leukocytes (PMNLs) - as well as cellular assay systems utilizing intact PMNLs. Furthermore, the antioxidant capacity of the extracts was evaluated using the ferric reducing antioxidant power (FRAP) - and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Following a comprehensive evaluation, it has been established that the methanol extract of golden flaxseed, obtained by Soxhlet extraction, was identified as the most effective of the extracts examined. These results suggest that golden flaxseed oil cake contains extractable compounds with relevant bioactivity, and that extraction conditions strongly influence their yield and functional profile. Further chemical characterization of the active fraction will be necessary to identify the compounds responsible for the observed effects and to evaluate their potential as anti-inflammatory lead structures or functional food ingredients.

POSTER 49

PROSTAMIDE/PROSTAGLANDIN F SYNTHASE NEGATIVELY REGULATES MYELINATION AND ADIPOGENESIS

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The enzyme prostamide/prostaglandin F synthase (PM/PGFS) catalyzes the conversion of PMH_2 to PMF_2alpha and PGH_2 to PGF_2alpha. We previously reported that PM/PGFS is constitutively expressed in the myelin sheaths of oligodendrocytes (OLs), suggesting its possible implications in myelinating OLs. In this study, we established PM/PGFS KO MO3.13 cells by CRISPR/Cas9-mediated genome editing to analyze the function of PM/PGFS in OLs. Rescued cells were constructed to re-express PM/PGFS in PM/PGFS KO MO3.13 cells. PM/PGFS KO cells showed increased expression of MBP, Olig2, CREB, and SOX10 proteins. In addition, the KO cells exhibited increased cholesterol synthesis and expression of SREBP-2, a key regulator of cholesterol synthesis. The KO cells also showed increased expression of the adipogenic factors, PPARgamma and Cebpa. These phenotypes were abrogated in the rescued cells. The addition of PMF_2alpha, a PMF_2alpha analog bimatoprost, PGF_2alpha, and PGF2alpha receptor (FP) agonists (latanoprost and travoprost) to MO3.13 cells decreased these myelin markers, adipogenic factors, and cholesterol level. The FP antagonist AL-8810 selectively antagonized the effects of PGF_2alpha, latanoprost, and travoprost but did not influence those of PMF_2alpha and bimatoprost. These data suggest that PMF_2alpha and bimatoprost interact with the points of action other than FP in MO3.13 cells. The present study demonstrates that PM/PGFS downregulates myelination and adipogenesis in OLs, suggesting that it may be a promising therapeutic target for demyelinating diseases including multiple sclerosis.