BOOK OF ABSTRACTS

9th European Workshop on Lipid Mediators

Edinburgh, June 26-28, 2024

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ORAL PRESENTATIONS

Educational Session - Bioactive lipids & NC-IUPHAR

Supported by the British Pharmacological Society

CHALLENGES IN IDENTIFYING RECEPTORS FOR LIPID MEDIATORS

Stephen Alexander

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Chair, Nomenclature and Standards Committee of the International Union of Basic and Clinical Pharmacology

A primary role of the Nomenclature and Standards Committee of the International Union of Basic and Clinical Pharmacology is to provide guidance on the nomenclature of drug targets that is clear, logical and consistent so that the broader scientific community avoids confusion and saves time/energy. These are published via the IUPHAR/BPS GuidetoPharmacology.org open access online database.

Subcommittees of NC-IUPHAR consider the nomenclature for specific receptors and families of receptors, with an aim to reflect the endogenous, 'canonical' ligand and to align subfamilies of receptors responding to the same endogenous ligand. Sometimes this has been straightforward and sometimes not.

A number of challenges arise in the process of naming 'orphan' G protein-coupled receptors, of which there are close to a hundred in the human genome. While a given receptor may respond to a given compound, we would not consider that sufficient evidence for changing a receptor name. At NC-IUPHAR, we consider a number of criteria to recommend a change in receptor naming. Relevant to the study of lipid mediators is consideration of the potency of the putative endogenous ligand/s and comparison with tissue concentrations. Additionally, whether there are plausible mechanisms (synthetic enzymatic pathways) in close juxtaposition to the receptors to allow generation of the putative endogenous ligand. Lipid derivatives provide a particular challenge as potential endogenous ligands for their heterogeneous distribution in biological systems, their bio/chemical lability and the thresholds for detection with analytical techniques.

LIPID MAPS

Valerie O'Donnell

College of Biomedical and Life Sciences, Cardiff, UK

LIPID MAPS (LIPID Metabolites and Pathways Strategy - link), was founded 20 years ago in the USA to systematically catalogue all lipids in mammalian cells, and support the developing field of lipidomics. A major contribution was establishment of a systematic and standardized approach for cataloguing lipid structural and biochemical data. To this end, the LIPID MAPS nomenclature and classification has become the globally used community standard. LIPID MAPS hosts databases containing lipids described at several levels of characterization as well as associated software tools and educational resources. LIPID MAPS became an ELIXIR-CORE data resource, and member of the Global BioData Coalition in 2023. This short talk will cover the key features of LIPID MAPS which include recent expansion of databases to incorporate richer metadata, literature provenance, taxonomic data and FAIR compliance. A joint project funded by ELIXIR-UK, in collaboration with WikiPathways, recently curated biochemical pathway data, and annotated lipids in context with their biochemical pathways. Search infrastructure updates have been implemented, along with enhanced programmatic access via API and SPARQL. New lipid-specific databases were developed and lipidomics tools provision enhanced. An extensive training and engagement programme includes webinars, podcasts and an online training school.

THE ROLE OF HEALING: THE OMEGA-3-DERIVED LIPID MEDIATORS PATHWAY

Cristina López-Vicario

Inflammation and Liver Disease group at IDIBAPS, Hospital Clinic-Ciberehd, European Foundation for the Study of Chronic Liver Failure (EF CLIF)

Historically, lipids have been associated with two primary functions: serving as structural components of membranes and providing a source of metabolic energy. However, a third function has emerged, involving the signaling and regulation of cellular function. Polyunsaturated fatty acids (PUFAs) are broadly classified into two families: omega-6 and omega-3. There is evidence indicating that lipid mediators derived from omega-3 and omega-6 PUFAs play a crucial role in the initiation and resolution of the inflammatory response. However, lipid mediators derived from omega-6 and omega-3 PUFAs have antagonistic effects and are competitively synthesized by the same metabolic pathway, meaning that an excess of one can be detrimental to the other. Therefore, the balance between omega-6 and omega-3 lipid mediators is essential for maintaining body homeostasis. Transgenic fat-1 mice, which express the *Caenorhabditis elegans* omega-3 fatty acid desaturase gene, represent a useful model to explore this balance. These mice have an abundant distribution of omega-3 fatty acids in their tissues from the embryonic stage and throughout their lives. This educational session provides new insights into the protective phenotype of transgenic fat-1 mice and the effects of omega-3 bioactive lipid mediators in the context of the chronic inflammatory state present in obesity and related metabolic syndrome.

1st Sponsored Session

Supported by GOED

(Global Organization for EPA and DHA Omega-3s)

OMEGA-3 FATTY ACIDS AND INFLAMMATION: FROM THE MEMBRANE TO THE NUCLEUS

Philip Calder

Faculty of Medicine, University of Southampton, UK

Inflammation is a normal part of the immune response and should be self-limiting (i.e. resolving). Excessive or unresolved inflammation is linked to tissue damage, pathology and ill health. Prostaglandins and leukotrienes produced from the n-6 fatty acid arachidonic acid are involved in inflammation and their production is a target for commonly used anti-inflammatory therapeutics. Fatty acids may also influence inflammatory processes through mechanisms not necessarily involving lipid mediators. The n-3 fatty acids EPA and DHA possess a range of anti-inflammatory actions. Increased content of EPA and DHA in the membranes of cells involved in inflammation has effects on the physical nature of the membranes and on the formation of signalling platforms called lipid rafts; this effect is linked to interference in the initiation of pro-inflammatory signalling pathways. EPA and DHA inhibit arachidonic acid metabolism which yields prostaglandins and leukotrienes involved in inflammation. EPA gives rise to weak (e.g. less inflammatory) analogues and both EPA and DHA are substrates for the synthesis of specialised pro-resolving mediators. Through their effects on early signalling events in membranes and on the profile of lipid mediators produced, EPA and DHA alter both intracellular and intercellular signals. Within cells, this leads to altered patterns of gene expression and of protein production. The net result is decreased production of inflammatory cytokines, chemokines, adhesion molecules, proteases and enzymes. The anti-inflammatory and inflammation-resolving effects of EPA and DHA are relevant to both prevention and treatment of human diseases that have an inflammatory component.

NEW INSIGHTS INTO THE BIOSYNTHESIS OF OMEGA-3 LONG-CHAIN POLYUNSATURATED FATTY ACIDS

Richard P Bazinet

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Docosahexaenoic acid (DHA) is abundant in the brain where it regulates cell survival, neurogenesis, and neuroinflammation. DHA can be obtained from the diet or synthesized from alpha-linolenic acid (ALA; 18:3n-3) via a series of desaturation and elongation reactions occurring in the liver. Our team has recently observed that DHA dietary DHA can downregulate its own synthesis from ALA, but the mechanism remains undetermined. Furthermore, we have observed that DHA can increase eicosapentaenoic acid (EPA) levels by inhibiting EPA elongation as opposed to retroconversion. In this talk I will describe our newly published paper that demonstrated that dietary DHA regulates DHA synthesis in the liver by inhibiting EPA elongation. First, we show by tracing 13C content (Î´13C) of DHA via compound-specific isotope analysis, that following low dietary DHA, the brain receives DHA synthesized from ALA. We then show that dietary DHA increases mouse liver and serum EPA, which is dependent on ALA. Furthermore, by compound-specific isotope analysis we demonstrate that the source of increased EPA is slowed EPA metabolism, not increased DHA retroconversion as previously assumed. DHA feeding alone or with ALA lowered liver elongation of very long chain (ELOVL2, EPA elongation) enzyme activity despite no change in protein content. To further evaluate the role of ELOVL2, a liver-specific Elovl2 KO was generated showing that DHA feeding in the presence or absence of a functional liver ELOVL2 yields similar results. An enzyme competition assay for EPA elongation suggests both uncompetitive and noncompetitive inhibition by DHA depending on DHA levels. To translate our findings, we show that DHA supplementation in men and women increases EPA levels in a manner dependent on a SNP (rs953413) in the ELOVL2 gene. In conclusion, we identify a novel feedback inhibition pathway where dietary DHA downregulates its liver synthesis by inhibiting EPA elongation.

METABOLISM, TRANSPORT AND FUNCTIONS OF DOCOSAHEXAENOIC ACID (DHA)

Takao Shimizu

National Center for Global Health and Medicine, Tokyo & Institute of Microbial Chemistry, Tokyo, Japan

Docosahexaenoic acid (DHA) is predominantly stored in esterified forms within membrane phospholipids—namely phosphatidylcholine, phosphatidylethanolamine, and plasmalogens - as well as in triacylglycerols and cholesterols (1). DHA, in its free form or when oxidised, produces a group of lipid mediators known as docosanoids, a member of SRM (2). Through the enzymatic action of acyl-CoA synthetase 6 (ACSL6), DHA is transformed into DHA-CoA, which is then incorporated into lysophosphatidic acid (LPA) to produce phosphatidic acid (PA), a precursor common to all glycerophospholipids and triacylglycerol. This crucial step from LPA to DHA-enriched PA is catalysed by AGPAT3, discovered by our group (referred to as LPLAT3, 3). AGPAT3 is ubiquitously expressed across all tissues, with notable abundance in the retina, brain, and testis. Both global and conditional knockout of AGPAT3 in mice lead to blindness, neuronal abnormalities, and male infertility, underlining the indispensable roles of DHA-phospholipids in physiological functions. It is particularly noteworthy that dietary DHA supplementation cannot rectify the retinal phenotypes, highlighting the unique role of endogenously produced DHA-phospholipids. Furthermore, a loss-of-function variant in AGPAT3 is associated with IDRP in humans (4). Maternal deficiency in DHA-phospholipids is correlated with behavioural abnormalities in genetically normal neonates and infants, even with sufficient dietary supplementation (5). ACSL6-deficient mice exhibit phenotypes that, whilst similar, are less severe than those of AGPAT3 KO mice (6, 7), indicating the crucial roles of both DHA metabolites and DHA-phospholipids in the physiological functions of these organs. Deficiency of DHA in various organs, due to either genetic mutations or malnutrition, triggers a compensatory response in the liver, characterised by upregulation of SREBP-related genes and an increase in PUFAs (8). In conclusion, our discovery of AGPAT3 underscores DHA's critical and unique roles in health and DOHaD.

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BRIDGING FROM OMEGA-3 SCIENCE TO CONSUMER USAGE

Elana Natker

GOED (Global Organization for EPA and DHA Omega-3s), Salt Lake City, UT, USA

The science behind omega-3s is compelling, but how can we best turn that knowledge into education and action? In this presentation, we will learn who consumers are most likely to listen to for health advice, why it's so difficult to have clear and concise communications about omega-3s, and how best we can make an impact so that omega-3 intake is increased worldwide.

2nd Sponsored Session

Supported by Pharma Marine

CHEMOENZYMATIC SYNTHESIS OF STRUCTURED AND ENANTIOSTRUCTURED TRIACYLGLYCEROLS CONSTITUTING EPA AND DHA

Gudmundur G. Haraldsson

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The synthesis of three types of structured triacylglycerols (TAGs) constituting a pure n-3 polyunsaturated fatty acid (PUFA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), along with a pure saturated fatty acid, is described. The first category involves traditional MLM (medium-long-medium) type structured TAGs possessing EPA or DHA in the 2-position of the glycerol backbone and a saturated medium chain fatty acid (MCFAs) (C6:0, C8:0, C10:0 or C12:0) located in the terminal 1,3-positions. The second structured TAG category includes reversed structured TAGs of the LML type, this time with EPA or DHA placed in the 1,3-positions and the MCFA in the mid-position. The third category is enantiostructured TAGs of an LMM type possessing EPA or DHA in either of the sn-1 (the S-enantiomer) or the sn-3 position (the R-enantiomer) along with a saturated fatty acid (16:0 or 18:0) in the remaining sn-2 and sn-3 (or sn-1) positions of the glycerol moiety. The chemoenzymatic syntheses were brought about by a highly regioselective lipase to control the regiopurity of the products using the saturated fatty acids activated as vinyl esters or the n-3 PUFAs activated as oxime esters in the case of the reversed structured TAGs. Enantiopure solketals were used as chiral precursors in the syntheses of the enantiostructured TAGs. A chemical coupling agent (EDCI) was employed to incorporate the n-3 PUFAs and the saturated fatty acids into the sn-2 position of the acylglycerol intermediates. Furthermore, syntheses of ABC type enantiostructured TAGs possessing three different fatty acids (one of them EPA or DHA) will also be discussed. The possible uses of such structured TAGs will be pointed out.

METABOLIC FATE OF DIETARY DHA FROM REGIO- AND STEREOSPECIFIC POSITIONS OF TRIACYLGLYCEROLS IN RATS

Baoru Yang[1], Yuqing Zhang[1], Mikael Fabritius[1], Gudmundur Haraldsson[2], Kaisa Linderborg[1], Haraldur Gudmunsson[2], Heikki Kallio[1], Professor Yumei Zhang[3]

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Long chain n-3 polyunsaturated fatty acids (n-3 PUFAs) have a wide range of health promoting effects. Long-term exposure to diet low in n-3 fatty acids may reduce the tissue levels of important n-3 PUFAs such as eicosapentaenoic acid [20:5(n-3), EPA] and docosahexaenoic acid [22:6(n-3), DHA], compromising the normal physiology and health. Previous research has shown that the bioavailability of n-3 PUFAs depends on the molecular structures of the lipids carrying these PUFAs. Moreover, the positional distribution of n-3 PUFAs in lipid molecules may vary among different natural sources. Currently there is limited knowledge on the importance of stereospecific position in dietary triacylglycerols (TAG) on the bioavailability of n-3 PUFAs. In this research, the first aim was to investigate the impact of deficient feeding on the profiles of fatty acids and phosphatidylcholines (PC) in different tissues and organs. The second aim was to investigate the effects of positional distribution of DHA in TAG molecules on tissue accumulation of DHA in rats, using regio- and stereospecifically structured TAGs. Male Sprague Dawley rats of normal n-3 status were fed with n-3 deficient diet supplemented with structured TAGs containing DHA at sn-1, 2 or 3 position and palmitic acid moieties at remaining positions (500 mg TAG/kg body weight per day) for four weeks. For comparison, groups receiving standard n-3 adequate feed AIN-93G as well as n-3 deficient diet with or without added tripalmitin were also included. Four-week n-3 deficient feeding decreased DHA levels of all tissues and changed in PC species, which were efficiently corrected by DHA supplementation. Overall, the bioavailability of DHA was high despite the position in TAG, increasing DHA levels in all the tissues and organs. Compared to sn-3 DHA feeding, feeding with sn-1 DHA resulted in significantly higher DHA level in TAG of the liver and plasma, whereas sn-3 DHA feeding led to highest DHA accumulation in visceral fat among the DHA groups. Our results indicate a slight difference in the metabolic fate of DHA between the two primary positions of dietary TAGs. This is the first study on the bioavailability of DHA from regio- and enantiopure TAGs.

OXIDATIVE STABILITY STUDIES ON EPA AND DHA IN REGIO- AND ENANTIOPURE TRIACYLGLYCEROLS

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2) Science Institute, University of Iceland, Reykjavik, Iceland

Docosahexaenoic acid (DHA) and eicosapentaenoic acids (EPA) are essential for health but easily oxidized. Yet the influence of their exact location (sn-1, sn-2, or sn-3) in triacylglycerols (TAGs) on oxidative stability has been thus far unknown. A major obstacle for oxidation stability studies has been the lack of regio- or stereopure model compounds.

This presentation describes the first studies comparing oxidative stability of DHA and EPA in regio- and enantiopure triacylglycerols, in the case of DHA also with and without chiral RRR- α-tocopherol. Headspace solid-phase micro-extraction with gas chromatography-mass spectrometry, liquid chromatography–mass spectrometry, and nuclear magnetic resonance spectroscopy were applied to study the oxidation behavior.

EPA was overall more oxidatively stable than DHA. EPA and DHA were most stable in the sn-2 position with or without added RRR-α-tocopherol resulting in differences in hydroperoxide formation.

Without the addition of antioxidant or in the presence of non-chiral antioxidant (butylated hydroxytoluene; BHT), oxidative stability of either EPA or DHA in sn-1 and sn-3 was almost similar with a tendency towards better stability at sn-3 in the propagation stage. With RRR-α-tocopherol higher stability of DHA in sn-1 compared to sn-3 was observed. This points to diastereomeric interactions between RRR-α-tocopherol and DHA in sn-1.

Additionally, the synthesized TAGs were submitted to static in vitro digestion (INFOGEST) for the first time. Also, in the gastrointestinal conditions, EPA at sn-2 was the most stable structure. Additionally, the location of DHA and EPA was seen to affect hydrolysis rates in vitro.

Overall, EPA and DHA are most oxidatively stable in sn-2 of triacylglycerols and oxidative stability in sn-1 and sn-3 is dependent on the chirality of antioxidant present. These results are highly relevant for enzymatic restructuring processes of DHA-rich fish or microalgae oil concentrates aimed for food supplements or food fortification.

Opening Plenary Lecture

NOVEL INSIGHTS INTO THE ROLE OF LIPID MEDIATORS IN CHRONIC LIVER DISEASE

Joan Clària

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Inflammation is a characteristic feature of virtually all chronic liver diseases. It intersects different liver pathologies from the early stages of liver injury, namely metabolic-associated steatohepatitis (MASH), when the inflammatory burden is mild-to-moderate, to very advanced stages of liver disease, specifically acute-on-chronic liver failure (ACLF), when the inflammatory response is very intense and drives multiple organ dysfunction and failure(s). The latter is characterized by an hyperinflammatory state that ultimately impairs the host defensive mechanisms of immune cells, rendering these patients immunocompromised and more vulnerable to secondary infections, and therefore to higher organ dysfunction and mortality. This presentation will update our current knowledge on the role of bioactive lipid mediators in the onset of the inflammatory process in these two different clinical entities across the liver disease spectrum (i.e. MASH and ACLF). Special emphasis will be given to gather the most relevant data on the role of pro-resolving lipid mediators that orchestrate the resolution of inflammation, a tightly controlled process which dysregulation might underlie the initiation and/or perpetuation of inflammatory processes in the setting of chronic liver disease.

Session 1

SPM RECEPTORS – GHOSTS OR REAL? NOVEL MECHANISMS OF ACTION

Mohamad Wessam Alnouri, Kenneth Anthony Roquid, Rémy Bonnavion, Haaglim Cho, Jan Heering, Gerd Geisslinger, Robert Gurke, Ewgenij Proschak, **Stefan Offermanns**

Max Planck Institute for Heart and Lung Research, Germany

Inflammation is a protective response to pathogens and injury. To be effective it needs to be resolved by endogenous mechanisms in order to avoid prolonged and excessive inflammation, which can become chronic. Specialized pro-resolving mediators (SPMs) are a group of lipids derived from omega-3 fatty acids, which induce the resolution of inflammation. How SPMs exert their anti-inflammatory and pro-resolving effects is, however, not clear. Here we show that SPMs such as protectins, maresins and D-series resolvins function as biased positive allosteric modulators of the prostaglandin E2 (PGE2) receptor EP4 through an intracellular binding site. They increase PGE2-induced Gs-mediated formation of cAMP and thereby promote anti-inflammatory signaling of EP4. In addition, SPMs endow the endogenous EP4 receptor on macrophages with the ability to couple to Gi-type G-proteins, which converts the EP4 receptor on macrophages from an anti-phagocytotic receptor to one increasing phagocytosis, a central mechanism of the pro-resolving activity of SPMs. In the absence of the EP4 receptor, SPMs lose their anti- inflammatory and pro-resolving activity *in vitro* and *in vivo*. Our findings reveal an unusual mechanism of allosteric receptor modulation by endogenous lipids and provide a mechanism by which SPMs exert their effects during the resolution of inflammation.

CHEMICAL PROBES TO STUDY LIPID SIGNALING IN MULTIPLE SCLEROSIS

Mario van der Stelt

Department of Molecular Physiology, Leiden University, The Netherlands

Signaling lipids, such as the endocannabinoids, play an important role in the brain. They regulate synaptic transmission and control various neurophysiological processes, including pain sensation, neuroinflammation, stress and anxiety. Unlike classical neurotransmitters, lipid messengers are produced on demand and degraded by metabolic enzymes to control their lifespan and signaling actions. Chemical biology approaches have become one of the main driving forces to study and unravel the physiological role of lipid messengers in the brain. In this presentation, I will discuss our program to study lipid metabolism in the brain of multiple sclerosis patients using chemical probes.

Punt JM, van der Vliet D, van der Stelt M. Chemical Probes to Control and Visualize Lipid Metabolism in the Brain. *Acc Chem Res*. **2022**;55(22):3205-3217.

NONCANONICAL FUNCTIONS OF 5-LIPOXYGENASE PATHWAY IN INFLAMMATION AND CANCER

Dieter Steinhilber

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5-Lipoxygenase (5-LO) is well known as key enzyme which catalyzes the initial two steps in the biosynthesis of leukotrienes from arachidonic acid. Leukotrienes are mediators of immune defense reactions and play a role in inflammatory and allergic reactions. Furthermore, the enzyme was shown to be involved in the development of certain types of cancer including different kinds of leukemia. Previous studies revealed that apart from its role in the generation of oxylipins, the enzyme modulates the activity of transcription factors such as p53 and beta-catenin via the regulation of their translocation into the nucleus.

In order to study the effects of 5-LO an gene expression, we generated a 5-LO knockout in the human monocytic MonoMac 6 cell line and performed genome-wide RNA-Seq analyses in undifferentiated and differentiated cells incubated with and without LPS. The data revealed that 5-LO regulates genes involved in cell adhesion, cell proliferation as well as a considerable number of NFkB response genes. Kynureninase and COX2 could be identified as 5-LO target genes. Previous data have shown that 5-LO is located within euchromatin regions in the nucleus. Using ChIP-Seq analyses, we can show that 5-LO is recruited to the promoter regions of its response genes and that it co-localizes with H3K27 acetylation, which is a marker for active enhancers. This noncanonical function of 5-LO is independent of its enzymatic activity as the function can be restored by knock-in of a catalytically inactive 5-LO mutant into the Mono Mac6 5-LO knockout cells.

Taken together, the data suggest that 5-LO has a noncanonical function to act as regulator of transcription factors involved in developmental and inflammatory pathways and that the development of 5-LO inhibitors that affect its noncanonical functions might be of therapeutic interest in the treatment inflammatory diseases and certain types of cancer.

RESOLUTION PHARMACOLOGY: A CHANGING PARADIGM IN THE TREATMENT OF INFLAMMATION

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The resolution of inflammation is a complete paradigm shift in the management of chronic inflammation. Now, the idea is not only blocking inflammation with anti-inflammatory drugs but activating the pathways of resolution. Indeed, inflammation end is not a passive mechanism due to the simple dilution or destruction of inflammatory mediators but genuinely an active mechanism linked to the synthesis of molecules that stop inflammation. These molecules are called lipoxins, maresins, resolvins and protectins and are grouped under the generic term of specialized pro-resolving mediators or SPM. SPM are a super family of ligands that activate receptors with 7 transmembrane domains and initiate signals to actively end inflammation. Dosage of SPM and set-up of good biological models are a key step in understanding those pathways. Discovery of a first- in-class monoclonal antibody promoting resolution and the role of inflammation in secondary lymphedema will here be discussed to put into perspective the development of new pro-resolutive therapies.

Session 2

ROLES OF OXYSTEROLS DURING NEUROINFLAMMATION: AN ATTRACTIVE STORY

Caroline Pot

Service of Neurology, Lausanne University Hospital, Switzerland

Perturbation of steroids pathways modulates inflammation and potentially a variety of diseases. Lipid metabolites have recently been ascribed functions as immune response modulators. In this line, oxysterols, oxidized forms of cholesterol, have pleiotropic roles on the immune responses aside from their involvements in lipid metabolism.

The oxysterols downstream the synthesizing enzyme cholesterol 25-hydroxylase (Ch25h), namely 25-hydroxycholesterol (25-OHC) and 7 alpha, 25-dihydroxycholesterol (7 alpha, 25-OHC), have recently been shown to regulate antiviral immunity and immune responses. In this presentation, I will present results obtained in my laboratory and discuss the multiple roles of Ch25h-derived oxysterols on adaptive immune cell differentiation and chemotaxis. I will further describe the implications of oxysterols during Multiple sclerosis (MS) and its animal model, the experimental autoimmune encephalomyelitis (EAE). MS is a chronic disabling disease of the central nervous system (CNS) commonly affecting young adults. The development and progression of this disease results in part from a disbalance between pathogenic effector T cells and negative regulation imposed by regulatory cells. We showed that Ch25h deficient mice display an attenuated EAE disease course. To further elucidate the cellular source of Ch25h during neuroinflammation, we generated a floxed reporter-ch25h knock-in mouse. We identified blood endothelial cells as a source of oxysterols during neuroinflammation and evaluated the impact of Ch25h endothelial-specific deletion during EAE development. Selective Ch25h deletion in blood endothelial cells of the CNS was sufficient to attenuate EAE thus revealing a central role of endothelial-derived oxysterols in attenuation of neuroinflammation. Altogether, those results highlight pro-inflammatory roles of Ch25h-derived oxysterols during neuroinflammation that I will discuss in this presentation.

LIPID MEDIATORS IN THE RESOLUTION OF NEUROINFLAMMATION: TOWARDS AN ATLAS OF INFLAMMATION-RESOLUTION IN THE NEUROIMMUNE AXIS

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Uncontrolled or unresolved neuroinflammation is associated with many widely occurring neuroinflammatory/neurodegenerative diseases, for which there is still an unmet need for diagnostic and therapeutic options. Indeed, in such diseases several cell autonomous or non-cell autonomous neuroinflammatory mechanisms play a role in the selective degeneration of affected brain areas, suggesting that both peripheral and central nervous system inflammation is important in their pathogenesis. These features could be a consequence of a failure to resolve inflammation and to restore tissue homeostasis. However, studies that link the immune system with inflammation resolution in neurodegenerative diseases is still unknown. Since inflammation resolution entails complex coordinated processes, involving numerous cell types interacting in space and time, using concepts of the Atlas of Inflammation resolution (AIR), we started to capture information on production of pro-inflammatory and pro-resolving lipid mediators as well as expression of the entire enzymatic machinery involved in biosynthesis and degradation of specialized pro-resolving mediators (SPMs) and their target receptors in a paradigm of neurodegenerative disease such as Parkinson's disease (PD), by analysing cerebrospinal fluid (CSF), plasma and peripheral blood leukocytes in patients at different clinical stages. By means of targeted LC-tandem MS-based lipidomics we identified unique lipid mediator signatures associated with clinical forms and we reported a higher presence of specific eicosanoids coupled with an impaired production of specific SPMs in both CSF and plasma. Such bioactive lipids were only induced in newly diagnosed de novo PD patients, whereas moderately advanced and severe PD patients showed levels below limits of detection. Furthermore, by means of high dimensional flow cytometry, we analysed enzymes and receptors involved in SPM metabolism and bioaction in 37 distinct immune cell subsets of both innate and adaptive and we identified disease-associated cell types responsible for peripheral immune dysfunctions in resolving inflammation. Overall, we here provide unprecedented evidence of a failed resolution pathway within the neuro-immune axis in PD, suggesting new insights into its pathogenesis and providing innovative diagnostic and therapeutic approaches.

IDENTIFYING A NOVEL GENETIC VARIANT ASSOCIATED WITH SANDHOFF DISEASE IN THE MOROCCAN POPULATION THROUGH TLC, HPLC MSMS, MOLECULAR NETWORKING, AND NGS-BASED GENETIC SCREENING COMBINATION

Miloud Hammoud[1], Alice M. S. Rodriguesc [2], Didier Stien [2], Emeline Houël [2], Naima Fdil[1]

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Sandhoff disease is a rare genetic disorder caused by a deficiency of the enzyme beta-hexosaminidase, leading to the accumulation of glycolipids such as GM2 ganglioside and globosides like Tetraglycosylceramide (Gb4).

The aim of this work is to identify and describe the particular biomarkers associated with a novel mutation of Sandhoff disease. In our study, we present an integrated analytical approach that merges Thin-Layer Chromatography (TLC), Ultra-High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UHPLC-MS/MS), and molecular networking.

Our study focused on 15 patients ranging from 10 months to 25 years old, exhibiting clinical symptoms associated with Sandhoff's disease, characterized by psychomotor regression, axial and peripheral hypotonia. We made a biochemical diagnosis by analyzing lipid extracts from patients' morning urine samples using thin- layer chromatography according to the Folch method and the individual globosides were subsequently characterized and quantified through HPLC MS-MS. We used molecular networking to the MS/MS data to identify lipid profiles specific to Sandhoff's diseases. Following next-generation sequencing (NGS) panel-based genetic screening, the analysis was carried out.

The detection of a wide range of non-hydroxylated globosides with saturated and unsaturated C16 to C24 fatty acyl chains, in two patients from the same family (1.5-year-old girl and her brother 8 months) likely indicates a disturbance in lipid metabolism associated with Sandhoff disease. The molecular network encompassed all types of globosides. The New missense variant c.1543A>C (p.Ser515Arg) located on the HEXB gene of Sandhoff disease was detected. Additionally, the remain thirteen patients suspected of having other neurodegenerative diseases showed elevated levels of globoside (GB3) and lactosylceramide evoking a pathological disturbance of lipid metabolism.

Combining TLC, UHPLC-MS/MS, and molecular networking offers a robust and precise analytical method to detect GB4 as biomarkers assigned to known pathologies and as biomarkers suggestive of new lipidosis. This comprehensive approach enhances not only, diagnostic accuracy and prognosis but also deepens our insight into the disease's mechanisms.

MODULATING MICROGLIA PHAGOCYTOSIS AFTER ISCHEMIC STROKE

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Ischemic stroke is one of the leading causes of death and disability in adults. Currently, there are no effective drugs to promote the functional recovery from stroke. Adequate healing of the damaged brain area depends on clearance of cell and myelin debris, but this process is slow and perturbs with neuronal regeneration. Thus, enhancing phagocytosis could improve removal of cell debris.

We have created a small library of lentiviral vectors (LVs) encoding different genes related to phagocytosis or recruitment of microglia/macrophages, namely Monocyte chemoattractant protein 1 (MCP1), three isoforms of Macrophage colony-stimulating factor (M-CSF) 1-190, 32-E, 1-E, Complement Component 3 (C3), Complement Component 3a (C3a), Adhesion G protein-coupled receptor E1 (Emr1/ADGRE1/F4/80), MER receptor tyrosine kinase (MerTK) and Mesencephalic astrocyte-derived neurotrophic factor (MANF). Their effect on phagocytosis and induction of inflammation were tested in microglia (BV2) after transient transfection in vitro by phagocytosis assay and cytokine (TNFa, IL-6 and IL10) ELISAs and the green fluorescent protein (GFP) was used as a control. Short, soluble peptides: MCP1, MCSF (1-190) and MANF were also tested in a transient distal Middle Cerebral Artery occlusion (dMCAo) model in rats where the effect on functional outcome, activation of microglia and the lipid mediator profile of plasma was determined.

The highest effect on phagocytosis in vitro was detected with LV-MerTK, LV-MCSF32-E and LV-MCSF1-E while LV-C3a and LV-Emr1 transfections enhanced phagocytosis over 80% of the induction of the positive control. In parallel, LV-MANF and LV-MerTK were equally potent in enhancing TNFa and IL-6.
All tested lentiviruses expressed the coded human proteins two weeks after ischemic stroke. Microglia proliferation was induced by MCP1 and MCSF 1-190 while MANF induced the microglia phagocytosis in the lesion area. However, there was no difference in regards of behavioural outcome and plasma oxylipin levels were mostly back to the baseline levels. Only PGE2 levels in all groups and TXB2 levels in MCSF and MANF groups were significantly reduced.

In summary, this is the first study to compare the effect of different chemotactic and phagocytosis related proteins enhancing phagocytosis and changing the inflammatory profile of microglia after stroke.

Session 3

IDENTIFICATION OF BACTERIAL LIPIDS AS KEY PLAYER IN VISCERAL PAIN

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Surveys on Western populations have estimated the irritable bowel syndrome (IBS) prevalence between 4-10% in adolescents and adults. IBS is characterized by abdominal pain associated with transit disorders. Even if the pathophysiology is poorly understood, IBS appears as a consequence of an alteration of the microbiota/gut/brain axis. The gut microbiota from patients with IBS, but not from healthy individuals, can induce a gut dysfunction in mice reminiscent of that seen in IBS suggesting that the microbiota contributes to IBS expression. In IBS, many studies investigated the taxonomy of the intestinal microbiota, but few studies have investigated active genes, proteins, or metabolites. Studies of molecules produced by the microbiota may be more relevant than its composition when it comes to understand the role played by microbiota in host function. In fact, the taxonomic composition of the human microbiome varies tremendously across individuals, while its functional capacity is highly conserved. This conserved functional capacity is defined as the functional redundancy of the microbiota. The general aim of our research is to identify metabolites differentially produced by the gut microbiota of IBS patients and to determine their effect on the host with a focus on visceral pain. Amongst bacterial metabolites, several lipids have been described for their capacity to cross the epithelial barrier. Based on our previous studies and others demonstrating the ability of lipids to regulate sensory neurons activation, we have decided to focus our attention on bacterial lipids in order to evaluate if they could link microbiota with IBS symptoms. Notably, we identified a new family of bioactive lipopeptides containing GABA produced by several bacteria of the microbiota capable of decreasing visceral hypersensitivity. We highlighted bacterial interactions for the metabolism of glutamine into GABA-lipopeptides. Using two different animal models of IBS, we evidenced the importance of microbiota composition on the efficacy of glutamine on visceral pain and revealed a responder and a non-responder profile in IBS mouse models as observed in patients. Our studies highlighted the importance of bacterial lipids in the communication between the intestinal microbiota and the host and particularly in regulation of neuronal activation.

SPLA2S: FROM ANTIBACTERIAL ACTIVITY TO ROLE IN SEPSIS, MALARIA, COVID-19 AND MICROBIOTA: QUO VADIS?

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Secreted phospholipases A2 (sPLA2s) constitute a family of structurally-conserved enzymes present in animal and plant genomes. They are particularly abundant in snake venoms and exist as a family of 11-12 isoforms in humans. It is now clear that the human enzymes are diverse in structure, tissue distribution and enzymatic properties, and play different roles in physiological and pathophysiological conditions, via mechanisms that are enzymatically-dependent or not, and also bind to proteins such as PLA2R1.

In this presentation, I will focus on the antimicrobial activity of sPLA2s against bacteria, viruses, parasites and fungi, up to applications in therapy and diagnosis. As early as late 70's, Elsbach group has shown antibacterial activity of human group IIA sPLA2. Venom and mammalian sPLA2s have in vitro and in vivo antiviral activities against viruses including HIV and other enveloped viruses, up to SARS-CoV-2. They also have antimalarial and antifungal activity. Finally, sPLA2s are involved in the control of gut microbiota, in symbiosis and dysbiosis. The sPLA2s act by various mechanisms, either directly against the pathogens, or indirectly, by modifying the immune response. Interestingly, bacterial strains have developed mechanisms of resistance against sPLA2 antibacterial activity.

These findings have led to medical investigations. sPLA2 active site inhibitors like Varespladib, initially developed by Eli Lilly Pharmaceuticals, have been tested in clinical trials in sepsis, but have failed. More recently, the drug has been repurposed for Covid-19. New specific sPLA2 inhibitors including antibodies are in development and remain to prove efficiency in infections or other diseases including cancers. sPLA2s may also be biomarkers of diagnosis and disease severity for infectious diseases. This includes sepsis, Covid-19, and beyond, including cardiovascular diseases.

I will finally discuss opportunities and concepts based on the molecular evolution of sPLA2s and their natural inhibitors. sPLA2s may be friends or enemies during infection, and we should inhibit the "bad ones", but not the "good ones". Last but not least, PLA2R1 has been identified as the major autoantigen in membranous nephropathy (MN), a rare but severe autoimmune kidney disease, and detection of anti-PLA2R1 autoantibodies has led to a paradigm shift in diagnosis and management of MN patients. In the next decade, sPLA2s may also become game changers in infectious diseases and beyond.

A REGULATORY LOOP INVOLVING A CYTOCHROME P450-SOLUBLE EPOXIDE HYDROLASE AXIS AND TGF-BETA SIGNALING DETERMINES PRO-RESOLVING POLARIZATION IN MACROPHAGES

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Polyunsaturated fatty acid metabolites generated by the cytochrome P450 - soluble epoxide hydrolase (sEH) pathway regulate inflammation, but little is known about their role in its resolution. In a model of zymosan induced peritonitis we observed a delay in the resolution of inflammation in sEH-/- mice. Using monocyte-derived macrophages we observed a marked increase in sEH expression following repolarization from a classically activated to pro-resolving phenotype with TGF-beta that required the activation of Alk5 and Smad2. However, sEH-deficient macrophages failed to fully repolarize, were less efficient at phagocytosis and retained a pro-inflammatory gene expression profile. Repolarization elicited marked changes in macrophage PUFA metabolites and 11,12-epoxyeicosatrienoic acid (EET) was able to reproduce the effect of sEH-deletion on gene expression. 11,12-EET was also found to attenuate the expression of Alk5 to inhibit the TGF-beta-induced phosphorylation of Smad2 by eliciting the translocation of the E3 ligase; Smurf2. These results indicate that the expression of sEH is not only controlled by TGF-beta but that the activity of the enzyme, which keeps levels of 11,12-EET low, actually promotes TGF-beta signaling by preventing the proteolytic degradation of Alk5. Thus, an autocrine loop between the sEH/11,12-EET and TGF-beta1 determines macrophage function.

PHOSPHOINOSITIDE ACYL CHAIN SATURATION DRIVES CD8+ EFFECTOR T CELL SIGNALING AND FUNCTION

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How lipidome changes support CD8+ effector T (Teff) cell differentiation is not well understood. We found that two separate phosphoinositide (PIPn) pools, marked by different acyl chain compositions, drive important signalling events at specific stages of Teff cell differentiation. Teff cell PIPn signaling was maintained by the rapid and continuous de novo saturated PI synthesis from glucose, directly tying the nutrient environment with Teff signaling. Saturated PI synthesis was increased in CD8+ TILs, and CDIPT, which catalyses the final enzymatic step in de novo PI synthesis, was required for CD8+ TIL antitumor fitness and function. T cells with improved antitumor function following checkpoint inhibitor therapy synthesized more saturated PIPn in mouse and human melanoma.

Plenary lecture

SPHINGOSINE-1-PHOSPHATE IN HEALTH AND DISEASES

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In this lecture I will present my adventures with the bioactive sphingolipid metabolite sphingosine-I-phosphate (SIP). My lab has been involved in the study of sphingolipids and sphingolipid metabolites as signaling molecules from their origin. Our research was and still is focused on the enigmatic lipid mediator, SIP whose role as a signaling lipid was discovered in my lab more than three decades ago. Our lab has uncovered many important physiological and pathophysiological processes important for inflammation, obesity, and cancer are regulated by SIP. We were the first to clone and characterize sphingosine kinases, SphK1 and SphK2, and SIP phosphatases, the enzymes that regulate SIP levels, providing molecular tools for the field. Our identification of the SIP family of GPCRs set the stage for the elucidation of their important functions. We also showed that SIP is a critical factor that influences cells' fate and developed the concept of the sphingolipid rheostat, and the paradigm of inside-out signaling by SIP. The puzzle of how such a simple molecule as SIP can have such diverse roles has been resolved by our finding that SIP functions not only as a ligand for SIP receptors, but also has important intracellular actions. I will discuss what we used to know about SIP and what we know now, describe well stablished concepts in SIP biology and actions. I will also highlight emerging and new concepts in SIP actions and describe evolving concepts from bench to clinic of targeting the SphK/SIP/SIPR axis that suggest that it is a useful therapeutic approach for several human diseases. Supported by NIH grant R35GM152058.

Session 4

ROLE OF LIPID MEDIATORS IN BROWN ADIPOSE TISSUE ACTIVITY IN OBESITY AND AGING

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Brown adipose tissue (BAT) activation has been proposed as a protective mechanism against obesity and related metabolic disorders such as type 2 diabetes. BAT activity is lower in obese subjects and decline with aging. BAT dysfunction in aging and obesity has been related to chronic unresolved inflammation, which could be mediated by an impaired production of specialized proresolving lipid mediators (SPMs). In a previous study[1], we demonstrated that arachidonic acid (AA)-derived lipoxins (LXs) and DHA-derived maresins (MaRs) and protectins (PDs) are the most abundant SPMs in BAT of young lean mice. Interestingly, the sum of SPMs is significantly lower in aged diet-induced obese (DIO) mice compared to young lean mice.

The identification of new therapeutic agents capable of activating BAT has been proposed as a strategy to tackle obesity and related metabolic diseases. n-3 polyunsaturated fatty acids (n-3 PUFAs) have been described as inducers of BAT activity and white adipose tissue (WAT) browning in mice. Other bioactive lipid metabolites derived from PUFAs such as PGI2, PGE2, PGF2alpha and the lipokine 12,13-DiHOME has been also identified as regulators of thermogenesis in BAT and beige adipocytes. More recently, our research group found that Maresin 1 (MaR1) activates BAT and promotes WAT browning in DIO mice, in parallel with increases in M2 macrophage markers[2]. Interestingly, the thermogenic properties of MaR1 are abrogated in IL6 knockout mice, suggesting that IL-6 is required for the thermogenic actions of MaR1. Moreover, the stimulatory effect of MaR1 on thermogenic genes observed in cultured brown adipocytes is abrogated in LGR6-depleted cells, suggesting that LGR6 receptor is mediating MaR1 actions on brown adipocytes. In summary, all these data support that MaR1 is a novel player that promotes BAT activation and WAT browning. These actions of MaR1 could contribute to its previously reported insulin sensitizing and antisteatotic properties, representing a promising therapeutic agent for obesity-associated metabolic disorders.

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Acknowledgements: MCIN/ AEI/10.13039/501100011033 (BFU2012-36089; BFU2015-65937-R; PID2019-106982RB-I00), cofinanced by the European Regional Development Fund (ERDF); Dept. of Health, Navarra Government (67/2015), and CIBEROBN (CB12/03/30002), ISCIII, Spain.

PATHWAY-ORIENTED PROFILING OF LIPID MEDIATORS IN BRAIN CORTEX: ROLE OF MARINE LIPIDS AND LINK WITH METABOLIC HEALTH

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The change on dietary patterns in Western countries has been associated to the huge increase of metabolic dietary-induced diseases occurring during the last 50 years. Remarkably, a similar trend is observable in the current risen of neurological disorders and cognitive impairment, thus become one of the most concerns for public healthcare systems. In this complex relationship, dietary fat seems to play a determinant role. Dietary fatty acids can directly affect the brain by passing the blood-brain barrier, being incorporated into the membranes and affecting membrane working, performance and signaling processes. And noteworthy, lipid metabolism within the brain modulate neuronal function and signal nutrient status, thus may affect the metabolism in key peripheral metabolic tissues. The actual diets have resulted in a strong intake reduction on the levels of omega-3 polyunsaturated fats (n-3 PUFA). Such reduction is being suggested as a key factor underlying the common risen of both, metabolic and cognitive disorders. This talk presents the effect of the intake of different dietary fats, included marine lipids rich in DHA which is the major n-3 PUFA in brain, in different metabolic conditions. Results illustrated the strong correlation between the pathway-oriented profiling of lipid mediators asociated to fat intake, in both brain cortex and metabolic tissues. N-3 PUFA intake drove an anti-inflammatory and antioxidant phenotype in brain cortex, in which Protectin PDx and specially, Maresin MaR1, seem to actively participate. Redox Proteomics finally helped to illustrate the pathways implicated.

The Spanish Ministry of Science and Innovation (PLEC2022-009385, RTI2018-095659-B-I00) and Xunta de Galicia (IN607B 2023/05) supported this work.

AN INFLAMMATION-REGULATED METABOLITE, ARACHIDONOYL-TAURINE, PROTECTS FROM DIET-INDUCED HEPATIC STEATOSIS AND FIBROSIS IN MICE

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N-acyl taurines (NATs) are biologically active conjugates of a fatty acid and taurine, which are conserved from crayfish to mice and humans. The biological functions of NATs have only begun to be studied, but different NAT species already have yielded promising results in stimulating GLP-1 secretion and lowering lipid absorption and fatty liver development.

Arachidonic acid (ARA) is a precursor for inflammation-regulating molecules, but the roles of ARA-NAT in inflammatory disease have not been studied. Using liquid chromatography-mass spectrometry (LC-MS), we find that plasma ARA-NAT increases with ARA supplementation and states of inflammation in both mice and humans, and with genetic disruption of its degradation by FAAH (FAAH S268D) in mice.

To investigate the effects of elevating ARA-NAT in metabolic fatty liver disease development, wildtype and FAAH S268D mice were fed a high fat, high fructose diet +/- ARA for 28 wk. ARA feeding caused biliary ARA-NAT to rise to 12 mM in the FAAH S268D mice, a value similar to that of traditional bile acids, showing conversion into ARA-NAT may be an important part of ARA metabolism and transport in times of excess.

Importantly, the ARA-fed FAAH S268D mice were protected from hepatic steatosis and fibrosis, liver damage, and systemic inflammation. Lipid uptake was higher in these livers, but accumulated triacylglycerol was lower. This improved hepatic steatosis was due to increased mitochondrial oxidation of fatty acids, measured by respirometry in liver sections and through use of labeled substrate in primary hepatocytes. Additionally, direct treatment of wildtype mice with ARA-NAT lowered markers of diet-induced inflammation in the liver. This works reveals ARA-NAT as a novel regulator of lipid metabolism and inflammation with potential as a target for treatment or prevention of fatty liver disease.

PROINFLAMMATORY ROLE OF 12(S)-HETE THROUGH THE ACTIVATION OF THE EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) PATHWAY IN HUMAN LEUKOCYTES: IMPLICATIONS IN OBESITY-ASSOCIATED INFLAMMATION

Esther Titos[1,2,3,4], Cristina López-Vicario[1,3,5], Mònica Romo[1], Mireia Casulleras[1] , Noelia Pérez-Romero[6], Ana Isabel Martínez-Puchol[2], Belén Sánchez[1,5], Roger Flores-Costa[1], José Alcaraz-Quiles[1], Marta Duran-Güell[1,5], Ainitze Ibarzábal[7], Juan José Espert[7], and Joan Clària[1,3,4,5]

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Background and Aim: The dysregulation of lipid metabolism, particularly the imbalance between omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) is widely recognized as a significant contributor to the chronic low-grade inflammation observed in obesity. In a recent study, we demonstrated elevated levels of arachidonic acid (AA) metabolites, notably those arising from 12-lipoxygenase activity, in the peripheral circulation of individuals with obesity. The current study aimed to elucidate the role of AA-derived 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE) as a pro-inflammatory lipid mediator contributing to sustained activation of the innate immune system in human obesity.

Methods: Experiments were performed in freshly Ficoll-isolated peripheral blood mononuclear cells (PBMCs) and the monocyte cell line THP1, which were incubated with synthetic 12(S)-HETE. ERK activation was assessed by western blot, the expression of platelet-associated markers was captured by exploiting previously collected microarray data (GSE71415) and gene expression was evaluated by real-time PCR in leukocytes and adipose tissue from subjects with obesity and control individuals. Adipose tissue platelet glycoprotein CD61 was assessed by immunohistochemistry and plasma cytokine levels were measured by fluorescent multiplex bead-based immunoassay.

Results: Elevated plasma levels of 12(S)-HETE in patients with obesity were associated with the presence of systemic inflammation, showing significant positive correlations with circulating cytokine levels including IL-6, IL-1alpha, and MCP-1. Notably, the expression of the 12-HETE-G-protein-coupled receptor 31 (GPR31) was significantly upregulated in PBMCs from individuals with obesity. Upon 12(S)-HETE (0.1 microM) treatment, human leukocytes exhibited an up-regulated expression of major pro-inflammatory cytokines, including IL-6 and IL-1beta. This stimulatory effect was associated with a time-dependent activation of ERK1/2 phosphorylation, which was mitigated by pre-incubation of leukocytes with the selective ERK inhibitor PD98059. Remarkably, CD61 immunostaining revealed an elevated presence of platelets in obese adipose tissue, despite platelet counts in the peripheral circulation were similar in obese and control subjects. This result aligns with elevated gene expression of 12-lipoxygenase and platelet-associated markers, including P-selectin and integrins (ITGAM, ITGAX, and ITGAV), within obese adipose tissue.

Collectively, these findings suggest that platelet infiltration and platelet-derived 12(S)-HETE, acting via a GPR31-ERK1/2-dependent pathway, play a crucial role in triggering low-grade inflammation in adipose tissuein human obesity.

Supported by Spanish Ministerio de Ciencia e Innovacion (PID2022-138970OB-I00), CIBERehd funded by the Instituto de Salud Carlos III and AGAUR (2021 SGR 01323), Generalitat de Catalunya.

YOUNG INVESTIGATOR SESSION

INVESTIGATING THE ROLE OF OXYSTEROLS IN INFLAMMATORY BOWEL DISEASE PATHOGENESIS: INSIGHTS FROM EX VIVO AND IN VIVO MODELS

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Inflammatory bowel disease (IBD) encompasses a group of serious disorders characterized by chronic inflammation in the digestive tract. While existing treatments offer some relief and improve morbidity, individuals with IBD often endure debilitating symptoms that profoundly impact their quality of life.

Over the past two decades, extensive research has shed light on a class of lipid mediators known as oxysterols [1]. Among other interesting properties, several oxysterols are involved in immunity and inflammation. Previous research, including from our group, has demonstrated altered levels of several oxysterols, such as 25-hydroxycholesterol and 7alpha,25-dihydroxycholesterol, in murine models of colitis and colonbiopsies from IBD patients [2].

In this work, we explored the involvement of four oxysterols — 25-hydroxy cholesterol, 7alpha,25-dihydroxycholesterol, 25-hydroxycholesterol-3-sulfate, and 4beta-hydroxycholesterol — in IBD pathogenesis using various models of intestinal inflammation (including colon explants; organoids; THP-1 derived macrophages; DSS-induced colitis in mice). We found that 7alpha, 25-dihydroxycholesterol and 25-hydroxycholesterol-3-sulfate significantly decreased cytokine production in colon explants from female mice with colitis, whereas their effects were not observed in those from male mice. Interestingly, we found that male and female mice exhibited distinct changes in oxysterol levels during

DSS-induced colitis, aligning with the reported different sensitivity to DSS-induced colitis found in male and female mice. Additionally, 7alpha, 25-dihydroxy cholesterol and 25-hydroxycholesterol-3-sulfate reduced the activation of THP-1 macrophages induced by LPS and IFNγ supporting their potential anti-inflammatory properties.

Growing evidence supports the involvement of the G protein-coupled receptor GPR183 in the chemotactic effects of 7alpha, 25-dihydroxycholesterol on immune cells, as well as in the formation of tertiary lymphoid organs during disease progression. Our data suggest that 7alpha,25-dihydroxycholesterol may initiate a signaling pathway leading to the suppression of the inflammatory response in macrophages. Our ongoing research aims to elucidate whether this pathway involves GPR183 activation.

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LEVERAGING OXYLIPIN PROFILING AS AN APPROACH FOR MACROPHAGE PHENOTYPE SPECIFICATION UPON BACTERIAL INFECTION IN VITRO

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Monocyte-derived macrophages (MDM) are heterogeneous cells of the innate immune system that play a pivotal role in orchestrating immune responses. Traditionally, MDM are broadly categorized into classically activated pro-inflammatory M1 or alternatively activated pro-resolving M2 phenotypes. Through metabolomic and transcriptomic profiling of MDM from different microenvironments, additional subtypes have been identified which vastly expand the M1/ M2 paradigm and question the validity of this rudimentary separation. Therefore, M2 are subdivided to address their respective role in resolution of inflammation (M2a), blunting of immune responses (M2b), efferocytosis (M2c), and angiogenesis (M2d). However, most of the research is limited to metabolic and proteomic profiling while the role of bioactive oxylipins as key regulators of inflammation produced by MDM subtypes is hardly addressed yet. To further explore the spectrumof human macrophage polarization, we used a mass spectrometry-based approach to delineate oxylipin profiles of established and novel MDM phenotypes upon bacterial infection. Moreover, by employing anti-inflammatory compounds like the GSK-3beta-inhibitor 6BIGOE and the glucocorticoid dexamethasone (Dex), we wanted to address the potential of compounds for MDM polarization and introduced novel MDM subtypes termed M-6B and M-Dex, respectively. In alignment with the oxylipin profile of M1, M2b and M2d produce substantial prostaglandins with however impaired release of leukotrienes. Interestingly, polarization with 6BIGOE leads to a similar oxylipin profile characterized by elevated PGF2alpha and 5-HETE similar as the M2c phenotype, while this similarity is probably attributed to induction of IL-10. Unexpectedly, M-Dex exhibits elevated levels of 15-HETE through 15-LOX-2 expansion compared to M2c, suggesting a divergence from the classical activated M2c phenotype. Moreover, we found that polarization of human MDM vastly alters their ability to engulf E. coli particles, with increased phagocytic ability among the M2 subtypes. While M2b exhibits the highest phagocytic activity among all subtypes, our investigations revealed that polarization with dexamethasone and 6BIGOE efficiently influences this capacity. Notably, M2c and M-Dex display similar phagocytic behavior in contrast to altered oxylipin profiles. In summary, our study highlights the pivotal impact of anti-inflammatory 6BIGOE and Dex in MDM polarization and underscores oxylipin profiling as a sensitive and comprehensive tool for MDM phenotype characterization.

EFFECT OF PROSTANOIDS ON ACUTE LUNG INFLAMMATION

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2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamide (AEA) are the two main endocannabinoids. Similarly to arachidonic acid, both can be oxygenated by COX-2 ultimately resulting in prostaglandin-glycerol esters (PG-Gs) and prostaglandin-ethanolamides (PG-EAs or prostamides), respectively.
Data published to date suggest that PG-Gs and PG-EAs can be considered as bioactive lipids in their own right. We have shown, for example, that PGE2-G increases LPS-induced macrophage (J774) activation, while PGD2-G decreases their activation and reduces DSS-induced colitis. A work by Lauren and colleagues showed that PGF2a-EA reduced crypt and mucosal tissue damage in a colitis model.
So far, few studies have investigated the effects of PG-Gs and PG-EAs in the pulmonary context, yet modulating the endocannabinoid system by blocking the hydrolysis of 2-AG and AEA has beneficial effects on lung inflammation.
Thus, we decided to investigate the effects of PG-Gs and PG-EAs in the context of acute lung injury (ALI). As the question remains whether they have distinct effects from classical prostaglandins (PGs) we conducted comparative studies in which the effects of PGD2, PGE2 and PGF2Î± were compared with those of the corresponding PG-Gs and PG-EAs.
We first studied the effects of these compounds, in vitro, on the activation of primary alveolar macrophages, neutrophils and PBMCs. In this context, cells were co-incubated with lipopolysaccharides (LPS, 100ng/mL) and the compounds for 8 hours for alveolar macrophages and PBMCs, and for 4 hours for neutrophils. In a second step, the effects of PGs, PG-Gs and PG-EAs were studied, after their intranasal administration, in a murine model of ALI induced by intranasal administration of LPS.
Our data highlight the beneficial effects of PG, PG-G and PG-EA derivatives on primary cell activation. These anti-inflammatory properties were not reproduced by all compounds in vivo. Indeed, our data suggest that PGD2 and PGF2a, as well as the corresponding PG-Gs and PG-EAs, have no effect on pulmonary inflammation under our conditions. Nevertheless, we show that the administration of PGE2-EA and PGE2-G appear to decrease major features of the disease.
These results, support the interest for future research aimed at elucidating the precise role of these endocannabinoid derivatives in ALI.

SEX-RELATED ROLE OF LEUKOTRIENES TO SPHINGOSINE-1-PHOSPHATE-INDUCED ASTHMA-LIKE FEATURES

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Asthma is a chronic disease of the airways. Furthermore, there is a clear sex disparity in asthma, as well as therapeutic efficacy in asthma varies between males and females. Sex hormones play a crucial role in shaping the differences in asthma prevalence between males and females during the transition from childhood to adulthood. Sphingosine-1-phosphate (S1P) has been identified as a significant contributor to asthma in preclinical and clinical settings. The sphingolipid metabolism is significantly affected in asthma and the S1P levels increase and correlate with the severity of the disease (1). Leukotrienes (LTs) are lipid mediators involved in asthma pathogenesis, and we have recently demonstrated sex disparities in LT biosynthesis and anti-LT pharmacology in inflammation (2,3,4). Thus, this study aims to investigate the role of S1P in airway hyperresponsiveness (AHR) and the interaction with the LT pathway in the lung. For this purpose, male and female BALB/c mice were sensitized to ovalbumin (OVA) or exposed to systemic administration of S1P. Bronchi and pulmonary tissues were harvested for functional and molecular studies. Part of the mice were pretreated with L-cycloserine, an inhibitor of sphingolipid metabolism, or LT biosynthesis inhibitor, MK886. BALB/c mice exposed to OVA or S1P display sex-related asthma feature development in favour of females coupled to a higher Th-2 immune response and LT production. This sex dimorphism was associated with significant differences in AHR, plasma IgE, and IL-5 pulmonary levels. L-cycloserine treatment inhibited all asthma features similarly to MK886 in OVA-sensitized mice in a sex-dependent manner. Further, the pretreatment with MK886 reduces S1P-induced asthma-like features only in females by reducing IgE plasma level and Th-2 cell recruitment. In conclusion, these results suggest the existence of a sex-dependent functional interaction between S1P and LT signalling and the molecular basis of a sex-tailored therapy in asthma.

1. De Cunto et al., Br J Pharmacol.2020. doi: 10.1111/bph.14861
2. Rossi et al., Pharmacol Res. 2019. doi: 10.1016/j.phrs.2018.11.024;
3. Cerqua et al. Pharmacol Res. 2020. doi: 10.1016/j.phrs.2020.104905;
4. Pace et al., J Clin Invest. 2017, doi: 10.1172/JCI92885

INVESTIGATING THE PROSTAGLANDIN E2/IL-22 PATHWAY IN ECZEMA

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Background: Eczema, the commonest inflammatory skin condition, is a clinically heterogeneous disease whose pathogenesis relies on the interplay between immune, genetic and environmental factors. It is caused by predominantly type 2 and 17 immune responses (involving Th2, Th17 and Th22 cells) as well as a defective terminal differentiation of keratinocytes. Having previously demonstrated that the bioactive lipid mediator, prostaglandin E2 (PGE2), promoted lymphocyte production of IL-22 from Th22 cells in a mouse model of eczema, we now wish to determine whether this occurs in eczema patients.

Methods: CD3+ T cells were obtained via cell separation from whole blood following donation by healthy volunteers and were stimulated using CD3/CD28 antibodies, cultured with/without PGE2 in order to study the effect of PGE2 on the numbers of IL-22 and IL-17 producing T cells. Using publicly available datasets, we studied the correlation between PGE2/IL22 gene expression for a range of diseases including eczema. Furthermore, following ethical approval we undertook RNA seq of skin biopsies (lesional and non-lesional) from our acute and chronic eczema patients to determine the pathways which define these subgroups.

Results: Fluorescence-activated cell sorting demonstrated that PGE2 significantly promoted increased differentiation into IL-22 and IL-17 producing CD4+ T cells compared to controls. Gene data mining studies from publicly available datasets showed that there was a strongly significant positive correlation between PGE2 and IL-22 pathway gene expression in inflammatory conditions including eczema, a finding we are investigating further within our RNA seq dataset. In our data, expression of pathways such as “cytokine activity” are mainly determined by inflammatory status (lesional vs. non-lesional) while the expression of extracellular matrix genes separates acute inflammation from chronic inflammation.

Conclusion: In summary, our results suggest that the PGE2 /IL-22 pathway plays an important role in eczema pathogenesis although further analysis of our RNA seq data and validation of our findings is required in order to more clearly define the subgroups within this heterogenous disease.

THE 5-LIPOXYGENASE-ACTIVATING PROTEIN (FLAP) DIFFERENTIALLY IMPACTS THE BIOSYNTHESIS OF SPECIALIZED PRO-RESOLVING MEDIATORS AND LEUKOTRIENES.

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Lipoxygenases (LOX) transform arachidonic acid (AA, C20:4) and docosahexaenoic acid (DHA, C22:6) into bioactive lipid mediators (LM) that comprise pro-inflammatory leukotrienes (LT) but also the anti-inflammatory specialized pro-resolving mediators (SPM) such as lipoxins and D-resolvins. The 5-LOX-activating protein (FLAP) provides AA as substrate for generating LT via 5-Lipoxygenase (5-LOX). Notably, 5-LOX is also involved in the biosynthesis of certain SPM, namely, lipoxins (LX) and resolvins, (RvD) implying a role of FLAP in SPM formation. Several FLAP antagonists were developed in the last three decades and have been evaluated for the therapy of asthma, COPD, arthritis, and cardiovascular disease in the clinics. Despite this exhaustive development the question how FLAP antagonists impact the biosynthesis of SPM is still under debate. Mechanistic investigations of FLAP antagonists had been mainly neutrophils or monocytes with focus on AA-derived LM formati on, especially LTs, neglecting effects on SPM production and DHA transformation. Here, we show that FLAP antagonism suppresses conversion of AA by 5-LOX to LT and LXA4, but not so the conversion of DHA to RvD5. We used M1- and M2-like monocyte-derived macrophages and conducted a screening of nine well-known FLAP inhibitors displaying their LM profile after challenging the cells with exotoxins from Staphylococcus aureus. All FLAP inhibitors reduced formation of 5-LOX-derived LTs but elevated formation of SPM from DHA, such as RvD5. Some FLAP antagonists even induced SPM formation in resting M2 macrophages. Intriguingly, in co-cultures of human neutrophils and platelets, FLAP antagonism abolished LXA4 formation but RvD5 levels were unaffected. In conclusion, FLAP antagonists suppress conversion of AA by 5-LOX to LTs and LXA4 but not the conversion of DHA by 5-LOX to RvD5, which should be taken into account for the development of such compounds as anti-inflammatory drugs.1

1: Dahlke P, Peltner LK, Jordan PM, Werz O. Differential impact of 5-lipoxygenase-activating protein antagonists on the biosynthesis of leukotrienes and of specialized pro-resolving mediators. Front Pharmacol. 2023 Aug 23;14:1219160. doi: 10.3389/fphar.2023.1219160.

EFFECTS OF OMEGA-3 ON HUMAN CORONARY VASCULAR TONE INDUCED BY NEUROTRANSMITTERS

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Background: Coronary artery diseases are characterized by chronic inflammatory status and endothelial dysfunction. This involves an increased production of neurotransmitters such as serotonin (5 HT) and acetylcholine. On the other hand, inflammation increases levels of pro-inflammatory lipid mediators such as PGE2 and TxA2. These changes are associated with effects on the vascular function by increasing vasoconstriction. Specialized pro-resolving lipid mediators (SPM), derived from omega-3 polyunsaturated fatty acids: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) play an active role in the resolution of inflammation. Recent results from our group show that DHA and metabolites (Resolvin D1, D5 and Maresin 1) reduce contractions of human coronary arteries (HCA) induced by PGE2 [1]. On the other hand, RvD5 and Mar1 production by human vagus nerve has been measured [2], their impact on the cardiac neuronal system remains unexplored.

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

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

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

Conclusion: Our preliminary results indicate that omega-3 may have an effect on neuronal control of HCA vascular tone, suggesting potential innovative therapeutic strategies.

[1] Bouhadoun et al. 2023, PMID 36528331
[2] Serhan et al. 2018, PMID: 30355784

5-LOX ACTIVATING PROTEIN: A NOVEL TARGET TO BOOST RESOLUTION OF NEURO-INFLAMMATION IN MS

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Multiple sclerosis (MS) is a devastating neurological disease and one of the most prevalent autoimmune disorders in the Western world. Phagocytes like macrophages and microglia play a dual role in lesion progression by promoting both inflammation and remyelination. The resolution of inflammation is regulated by bio-active lipid mediators (LMs), that are produced by enzymes such as lipoxygenases (LOX). We observed that, in MS lesions 5-LOX expression was present in all CNS cell types. Interestingly, 5-LOX activating protein (FLAP) was increased locally in active lesions and was mostly confined to microglial cells suggesting that activation of the 5-LOX pathway through FLAP under inflammatory conditions is responsible for a detrimental LM shift possibly causing impairment of resolution and enhancing lesion progression. We now found that pharmacological inhibition of FLAP halts myelin induced foam cell formation and reduces proinflammatory cytokine production in LPS activated microglia in vitro. In line with these results, we show that FLAP inhibition promotes remyelination in a microglia-dependent manner and reduces the disease score in well-established ex vivo cerebellar brain slice and in vivo experimental autoimmune encephalomyelitis models.

Altogether, obtained results identified FLAP as a novel target to restore the LM balance, thereby preventing neuro-inflammation and promoting repair in MS and other neurological diseases characterized by the presence of chronic inflammation and demyelination.

INSIGHTS INTO PGE2-MEDIATED EP4 RECEPTOR ACTIVATION EXPLORED BY MOLECULAR SIMULATIONS

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Lipid mediators, such as prostaglandin E2 (PGE2), play fundamental roles in cellular communication and in the development of diseases like cancer. The lipid mediators interact with specialized proteins called G
protein-coupled receptors (GPCRs), with Prostaglandin E2 Receptor Type 4 (EP4) being particularly significant in cancer and inflammation, driving tumor growth, metastasis, and immune evasion. By employing advanced Free Energy Perturbation (FEP) and atomistic Molecular Dynamics (MD) simulations of the receptor-G proteins complex in its native membrane, we study here howPGE2 activates EP4, offering novel molecular insight into the treatment of diseases. In this regard, we unravel the complex interactions between PGE2 and EP4, revealing mechanisms behind receptor and G protein activation process. Our combined findings provide insights into the functional dynamics of for GPCRs for the future development of innovative therapeutic strategies for cancer by targeting key cellular signalling pathways.

Session 5

A LIPIDOMIC DISSECTION OF HOST-GUT MICROBIOTA-DIET TRIAD

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Symbiotic gut microbiota complex produces metabolites with high structural and functional variety, of using host dietary factors as building blocks. We have investigated unique alpha-galactosylceramides from the human symbiont *Bacteroides fragilis* (BfaGCs), playing a unique role in intestinal immune development and regulation. Even though multiple gut Bacteroidales produce several subclasses of sphingolipids, the biochemical capability to produce BfaGCs is restricted to very few species, and *B. fragilis* is the dominant contributor. Two *B. fragilis* enzymes, alpha-galactosylceramides (agcT) and branched-chain amino acid aminotransferase (bcaT), are essential components to produce bioactive BfaGCs. Furthermore, targeted deletion of either gene blunted bacterial regulation of colonic type I natural killer T cells in murine neonates. In accordance with animal model results, a high abundance of *B. fragilis* in human infants and a correlation with immune diseases were observed in multiple metagenomic studies implying a potential significance of BfaGCs in human immune development. We present a structural- and molecular-level paradigm of immunomodulatory control by endobiotic (gut symbiont-originated) metabolites through investigating dietary/microbial/immune system interdependence.

CHARACTERISATION OF THE VIRAL LIPID ENVELOPE OF PANDEMIC SARS-COV2 STRAINS, AND HOW IT'S IMPACTED BY INFLAMMATION.

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Little research has been conducted into the composition of virus lipid envelopes, in particular what lipids they contain and how these may be regulated by inflammation in host cells during viral replication. Most if not all studies (conducted back in the 1980s and 1990s) used traditional methods and so far, profiling of molecular species using mass spectrometry hasn't been performed for any enveloped virus. Here, recent studies from our laboratory will be presented that compares the lipidomes of SARS-CoV2 pandemic strains and how it responds to the inflammatory status of host cells. Knowledge of the lipid envelope composition of viral envelopes may reveal novel roles for the membrane in driving virus replication and associated inflammation, and could also aid in design of membrane disrupting agents of potential clinical utility.

BIOANALYTICS OF SPECIALIZED PRO-RESOLVING MEDIATORS: ADDRESSING VALID CRITICISM WITH VALIDATED ANALYTICAL TECHNIQUES

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Critics have challenged the occurrence and significance of tri-hydroxylated oxylipins and other specialized pro-resolving mediators (SPM) in biological samples by disputing the validity of SPM quantification methods. In response, the field of inflammation resolution science has re-evaluated the existing data and developed new, guideline-conforming approaches capable of providing reliable evidence of SPM in vitro and in vivo.

Two years after the initial critique was published [1], the reluctance to share primary data on SPM detection continues to spark controversy within the field of oxylipin research. To shed new light on the current state of SPM research, a summary of existing oxylipin quantification protocols is provided, correlating the degree of validation efforts of these methods with the biological levels of SPM reported thereby. Primary chromatograms and baseline-levels of representative in vivo and in vitro samples obtained by our own protocol for the identification and quantification of oxylipins using RPLC-MS/MS are made transparent. We demonstrate (1) pitfalls of validation regarding the lower limit of quantification (LLOQ) based on the commonly cited signal-to-noise ratio; (2) the tools for the robust detection of di-hydroxylated SPM (e.g. RvD5, PDx) but also tri-hydroxylated SPM (e.g., LXA4, RvE1) in primary human leukocytes well above the LLOQ of commonly employed (mid-range) tandem mass spectrometers; (3) the difference between commercially available maresin 1 (MaR1) and the macrophage-derived biological product commonly misinterpreted (including by us in the past) as MaR1.

By acknowledging the basis in fact of prior criticism directed at quantitative SPM methods, including the need for higher transparency and stricter validation, we shall create the common ground between advocates and critics of SPM-based resolution immunology needed to move the conversation towards the shared goal of optimizing oxylipin analysis.

[1] Critics challenge data showing key lipids can curb inflammation. Science 2022. doi:10.1126/science.abq8439.

LIPID MEDIATORS IN A NEW DIMENSION: 2D MULTIPLE HEART-CUTTING LC-MS/MS FOR THE QUANTITATIVE AND ENANTIOSELECTIVE ANALYSIS OF OXYLIPINS

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Quantitative analysis of oxylipins is key to the understanding of their biological role and their modulation by pharmaceutical drugs. Physiological effects of lipid mediators are rarely caused by individual oxylipins, but rather by a change in the oxylipin pattern. Therefore, liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods, covering > 150 structurally diverse oxylipins are state of the art.

These reversed-phase (RP) LC methods are characterized by high sensitivity and selectivity, which is achieved in particular by narrow chromatographic peaks and sensitive and selective detection by MS/MS.

Among the large number of oxidation products of different fatty acids, the enzymatic formation of oxylipins is regio- and stereoselective. For example, the human 5-lipoxygenase only gives rise to 5(S)-hydro(pero)xy-arachidonic acid. However, RP applications do not allow the separation of enantiomers, which is key to elucidate the formation route of oxylipins in biological samples.

Here, we developed the first two-dimensional (2D) LC method for the enantioselective analysis of oxylipins: The fatty acid metabolites are separated in the first dimension by highly efficient RP chromatography [Anal Bioanal Chem. 2023, 415(5), 913], separating positional isomers and in particular isobaric oxylipins. This minimizes interferences in the second dimension. By means of multiple heart-cutting, the peaks of the oxylipins are collected from the first dimension and transferred onto a chiral stationary phase. This amylose-based stationary phase allows efficient separation of 45 enantiomeric hydroxy- and dihydroxy-fatty acids with a generic linear gradient of water and acetonitrile within a second dimension cycle time of 1.80 min.

The method is suitable for determining oxylipin concentration as well as enantiomeric ratios in biological samples such as plasma and cell cultures. Furthermore, enantiomers and diastereomers of multiple hydroxylated fatty acids are separated, which is not possible with RP stationary phases. With this, we could show that dihydroxylated fatty acids found in human M2-like macrophages are formed by 5- and 15-LOX catalytic activity, and not predominantly by autoxidation.

Of note, this method can easily be used as an extension of existing comprehensive targeted metabolomics platforms and clinical samples can be analyzed for enantiomeric ratios from the same sample without additional sample preparation steps. The generated data sets provide comprehensive insights into the pathways of oxylipin formation and their role in the regulation of physiological processes such as inflammation.

CHECKPOINT INHIBITOR THERAPY, CHEMOTHERAPY AND LIPID MEDIATORS

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Immune checkpoint (ICI) therapy has an increasingly important role as antitumor therapy for various tumor entities. A recent study has shown that an increase of omega-3 fatty acid content as well as inhibition of the soluble epoxide hydrolase can enhance ICI efficacy in animal models.

In our previous work in the context of sorafenib therapy for hepatocellular carcinoma we were able to show increased levels of epoxyeicosanoids with sorafenib therapy.

As part of an on-going study we are assessing effects of cancer treatment regimens for lung cancer consisting of chemotherapy as well as immune checkpoint inhibitor therapy on blood content of lipid mediators.

Blood sampling took place before and 24 h after immune checkpoint therapy application. In a group of 21 patients we found that concentrations of CYP450 products increased after ICI: We found a significant increase of several arachidonic acid-derived epoxyeicosatrienoic acid (EET) levels, with the most pronounced increase observed for 14,15-EET, and several significantly increased docosahexaenoic acid-derived epoxydocosapentaenoic acids (EDPs). This is in contrast to our initial observations in a smaller set of patients, in which we found decreased EET and EDP levels after combined chemo- and ICI-therapy.

POSTER PRESENTATIONS

POSTER 1

JANUS KINASE INHIBITORS ENHANCE PROSTANOID BIOSYNTHESIS IN HUMAN WHOLE BLOOD IN VITRO: IMPLICATIONS FOR CARDIOVASCULAR SIDE-EFFECTS AND PREVENTION STRATEGIES

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Background: Janus kinase inhibitors (JAKis) are used to treat inflammatory diseases, e.g., rheumatoid arthritis (RA). However, tofacitinib, a JAKi, has been associated with an increased risk of cardiovascular (CV) side effects. The mechanisms behind these side effects are still unknown.

Objectives: This study aims to investigate the impact of JAKis on the biosynthesis of thromboxane A2 (TXA2) from platelets in response to thrombin and pro-inflammatory prostaglandin E2 (PGE2) and TXA2 from leukocytes in response to the inflammatory stimulator lipopolysaccharides (LPS) in human whole blood (HWB).

Methods: Four JAKis, tofacitinib, baricitinib, filgotinib, and upadacitinib , were investigated at 0.04-20 μM for their impact on the biosynthesis of platelet TXB2. HWB from healthy controls (HCs, n=12) was allowed to clot for 1 hour at 37°C, and serum TXB2 was detected by a validated enzyme immunoassay (EIA). The JAKis were also studied in LPS-stimulated heparinized whole blood collected from HCs (n=17), patients with systemic lupus erythematosus (SLE, n=12) or treatment-naïve axial spondyloarthritis (axSpA, n=12), after 24-hour incubation. PGE2 and TXB2 were assessed by liquid chromatography-mass spectrometry (LC-MS/MS).

Results: The serum levels of TXB2 were significantly enhanced by the 4 JAKis. At 4μM, tofacitinib, baricitinib, and upadacitinib increased serum TXB2 by 36±54, 39±65, and 31±46% (mean±SD, n=12), respectively, compared to vehicle control. At 0.04 μM of filgotinib, it was 44±49%. The addition of exogenous AA did not further increase serum TXB2. The antiplatelet agent aspirin (acetylsalicylic acid, ASA, 100μM) inhibited serum TXB2 synthesis (96±2%), and this effect was not changed in the presence of JAKis.
In the LPS-stimulated HWB, tofacitinib (1 μM) increased both PGE2 (48% ± 36%, mean±SD, $P<0.01$, n=17) and TXB2 (42% ± 33%, $P<0.01$, n=17) in HC. This increase was also seen in patients with SLE (PGE2: 89%
± 127%, $P<0.01$, n=12; TXB2: 57% ± 39%, $P<0.01$, n=12) and axSpA (PGE2: 50% ± 56%, $P<0.01$, n=12; TXB2: 29% ± 25%, $P<0.01$, n=12).

Conclusion: JAKis-induced dysregulated prostanoid biosynthesis in platelets and leukocytes may increase the risk of thromboinflammation in patients with chronic inflammatory disorders. Administration of low-dose aspirin may help mitigate the risk. However, further clinical studies are required to confirm our findings.

POSTER 2

mPGES-1 INHIBITOR ENHANCES THE CYTOTOXIC EFFECT OF VINCRISTINE IN PDAC MULTICELLULAR TUMOR SPHEROIDS

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The stroma surrounding the tumor is usually characterized by the presence of fibroblasts. Cancer cells can activate fibroblasts to perform various functions, ultimately leading to a tumor microenvironment that actively promotes tumor growth. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX) enzymes, resulting in decreased synthesis of prostaglandins (PGs). This not only has an anti- inflammatory effect, but also reduces tumor growth by inhibiting the synthesis of the tumor-promoting factor prostaglandin E2 (PGE2) and thus its signal transmission via prostaglandin E2 receptors (EP1-4). To avoid various adverse effects of COX inhibitors associated with non-selective PG reduction, inhibition of microsomal PGE synthase-1 (mPGES-1), which specifically blocks PGE2 formation, could be an interesting target. In this study, we investigated the effect of inhibiting mPGES-1 in combination with the cytotoxic drug vincristine on multicellular tumor spheroids (MCTS). We tested the effect of this combined treatment on pancreatic ductal adenocarcinoma (PDAC) cells (PaTu-8988T and Capan-2) co-cultured with human dermal fibroblasts (HDF) in MCTS models. Cell viability was assessed using an ATP-based cell viability assay and high-affinity nucleic acid staining. Combined treatment with mPGES-1 inhibitor 934 and vincristine resulted in significantly increased cell death in both PaTu-8988T and Capan-2/HDF MCTS compared to mono-treatment with vincristine. In addition, we tested combination treatment with celecoxib, EP2 and/or EP4 antagonists in both types of MCTS. The combination of vincristine and celecoxib or EP antagonists did not result in significantly higher cell death compared to vincristine treatment alone. We optimized Capan-2/HDF MCTS for high-throughput screening and advanced cell imaging analysis to thoroughly investigate the combination of mPGES-1 inhibitors with various other cytotoxic drugs. The initial results confirm previous findings that inhibition of mPGES-1 enhances the efficacy of vincristine, so the use of mPGES-1 inhibitors could be a new therapeutic approach for PDAC.

POSTER 3

UNLOCKING THE POTENTIAL OF OMEGA-3 FATTY ACIDS: MODULATION OF VASCULAR TONE IN PULMONARY HYPERTENSION

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Pulmonary hypertension (PH) is a severe disease that arises from multiple etiologies, leading to right ventricular failure and death.
Pulmonary perivascular inflammation has gradually gained increased attention as an early common hallmark across different PH groups. Most of the used treatments target the pulmonary vasoconstriction (PGI2 analogous, ET-1 inhibitors and Phosphodiesterase inhibitors) that stimulate smooth muscle cell relaxation. However, pulmonary hypertension remains associated with significant morbidity.
We therefore hypothesised that inflammation plays a crucial role in the severity of abnormal vasoconstriction in PH. Based on this hypothesis, we have selected a candidate family of bioactive lipids: omega-3 fatty acid (EPA, DHA and DPA) and their metabolites with high resolving potential, called the SPM for specialised proresolving mediators (resolvins, protectins and maresins).

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

In summary, our findings indicate that omega-3 fatty acids, and their metabolites the SPM possess inherent vasorelaxant properties, especially in non-PH HPA. However, in the context of PH, these relaxing effects seem to diminish. Additionally, our investigations unveiled intriguing interactions between omega-3 fatty acids, notably DHA and DPA, with HPA responses to Iloprost (enhancement of vasorelaxation) and Prostaglandin E2 (reduction of vasoconstriction) for both non-PH and PH HPA.

POSTER 4

CHARACTERIZATION OF P2Y/X RECEPTORS AND THEIR DYSFUNCTION IN THE RELAXATION OF PULMONARY ARTERY DERIVED FROM PATIENTS WITH OR WITHOUT PULMONARY HYPERTENSION OF GROUP 3

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

In this study, we evaluated the vasorelaxant or contractile responses of different nucleoside phosphates (ATP, ADP, UTP, UDP, ATPγS, Adenosine) in HPAs derived from PH Group 3 and Non-PH patients in the presence or absence of endothelium. A pharmacological study (organ bath system) was performed using selective purinergic receptor (P2Y/X) antagonists or agonists [2]. Moreover, we examined the contribution of nitric oxide (NO) and prostacyclin (PGI2) pathways to ATP-induced relaxation of HPAs using inhibitors of these pathways (L-NOARG and indomethacin, respectively).

This study showed that ATP, UTP and ATPγS produced greater relaxations of HPAs than ADP, UDP or Adenosine. The relaxant responses were mostly blocked by eitherMRS2279 (P2Y1), ARC118925 (P2Y2), ATP (10 µM, P2Y4) or AR-C118925 (P2Y2) while MRS4062 (P2Y4 agonist) was inactive. In addition, on HPAs derived from Group 3 PH patients, ATP and ADP produced a less potent relaxation (~ 50 % less compared to control). UTP and UDP did not produce any relaxation, suggesting a uridine purinergic dysfunction on HPAs derived from Group 3 PH patients.

Together, our results indicate that the purinergic agonists inducing-relaxation are endothelium, PGI2, and NO dependent. Moreover, we are currently able to identify two (2) purinergic receptors (P2Y1, P2Y2) involved in the relaxation, and we are currently investigating three (3) others (P2Y11 P2Y6, P2Y4). Finally, our results show that these relaxations are significantly reduced in HPAs from Group 3 PH patients, particularly the UTP relaxation, probably due to P2Y2 and P2Y4 dysfunction and might be a possible pathological mechanism underlying Group 3 PH.

[1] PMID : 32867554 / [2] PMID : 32037507

POSTER 5

ELUCIDATION OF THE OXYGENATION CAPACITY OF FREE AND MEMBRANE-BOUND FATTY ACIDS FROM CELLS HIGHLY EXPRESSING 15-LIPOXYGENASE ISOFORMS

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Oxylipins play pivotal roles in inflammation, driving acute and chronic inflammation, but also its resolution. When exposed to physiological and pathological stimuli, polyunsaturated fatty acids (PUFA) can be liberated from membrane phospholipids. These free PUFA can be converted into oxylipins via cyclooxygenases (COX-1 and -2) and lipoxygenases (5-, 12- and 15-LOX enzymes) and, in some cases, via specific additional downstream enzymes. While the COX pathway yields proinflammatory prostaglandins and the 5-LOX pathway proinflammatory leukotrienes, several anti-inflammatory oxylipins, e.g. lipoxins and resolvins, are formed primarily by 15-LOX-1/2 as key enzymes.
Interestingly, 15-LOX isoforms are unique in their ability to oxygenate esterified PUFA in phospholipids of biomembranes, in addition to free PUFA. Such enzymatically oxidized phospholipids are proposed to possess immunomodulatory activities, such as shaping the responsiveness to pathogen-associated molecular patterns (PAMPs) recognition receptors and orchestrating the clearance of apoptotic cells (efferocytosis). Nevertheless, the quantitative determination of oxylipins incorporated into membrane phospholipids is still an enormous technical challenge. We followed an approach to differentially measure on one hand free oxylipins upon cell incubation, and on the other hand total oxylipins after incubation using alkaline hydrolysis to additionally liberate esterified oxylipins from various lipid fractions adapting a published protocol1. Oxylipins were analyzed by UPLC-MS-MS. We tested hydrolysis efficiency under different conditions (KOH concentration and saponification time) and validated oxylipin recovery by using selected internal standards for the optimized protocol (0.2 M KOH at 37Â°C for 30 min). As suitable cellular models, we employed HEK293 cells and different polarized human monocyte-derived macrophages with high expression of 15-LOX-1/2 isoforms, and studied their biosynthetic capacity to form free and total oxylipins. We observed that esterified oxylipins, in addition to free oxylipins, are formed under all tested conditions (non-stimulated, bacterial toxins, curcumin) with varying degrees. We hypothesize that esterified oxylipins may mediate, at least in part, some effects accounted to 15-LOX enzymes in cells stimulated with bacterial- and plant-derived natural products.

1 Quehenberger et al. (2018). Quantitative determination of esterified eicosanoids and related oxygenated metabolites after base hydrolysis. Journal of lipid research 59, 2436-2445.

POSTER 6

SYNTHETIC STUDIES TOWARDS 4S,5S-DIHYDROXY DOCOSAPENTAENOIC ACID

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The polyunsaturated fatty acid (PUFA) product 4S,5S-dihydroxy docosapentaenoic acid (DPA) has been isolated from various human samples and is most likely enzymatically formed. This endogenous product has not yet been the target of a stereoselective total synthesis.

This poster presents some of the lessons learned during our attempted synthesis of 4S,5S-dihydroxy DPA, starting from commercially available docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). This approach has successfully been used earlier for other PUFA-derived natural products.

POSTER 7

SREBP2-DEPENDENT MACROPHAGE CHOLESTEROL HOMEOSTASIS UNDER THE CONTROL OF ALOX15B

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Increasing evidence links cholesterol biosynthesis and propagation of lipid peroxidation. In primary human macrophages, the lipid peroxidizing enzyme arachidonate 15-lipoxygenase type B (ALOX15B) is constitutively expressed and has been implicated in the regulation of cholesterol homeostasis. Global transcriptome analysis of ALOX15B-silenced macrophages pointed to a massive reduction of sterol regulatory element-binding protein (SREBP) 2, the master transcription factor of cellular cholesterol biosynthesis, as well as almost all its target genes. In line, immunofluorescence analysis confirmed that the knockdown of ALOX15B decreased the amount of nuclear localized SREBP2. In addition, reduced levels of the biosynthetic intermediates desmosterol and lathosterol, as well as the cholesterol-derived oxysterols 25- and 27-hydroxycholesterol indicated an attenuated flux through the cholesterol biosynthesis pathway in ALOX15B-depleted cells. Silencing ALOX15B decreased total cellular lipid peroxidation and LC-MS/MS analysis revealed the reduction of the lipid hydroxides 15-(HETE)hydroxy eicosatetraenoic acid, 15-(HEPE)hydroxyeicosapentaenoic acid and 13-(HODE)hydroxyoctadecadienoic acid in ALOX15B knockdown macrophages. Using the glutathione peroxidase (GPX) 4-inhibitor Ras-selective lethal (RSL) 3, we found that lipid peroxide accumulation increased SREBP2-dependent gene expression, whereas the free radical scavenger liproxstatin-1 decreased SREBP2 activity in macrophages. Finally, global transcriptome and Western analysis revealed that the knockdown of ALOX15B limited activation of the mitogen-activated protein (MAP) kinase ERK1/2, which we found to be sensitive to cellular levels of lipid peroxides. At the same time, inhibition of ERK1/2 decreased nuclear and cytosolic SREBP2 and in turn, the expression of SREBP2 target genes. A sterol-dependent inactivation of SREBP2 was ruled out since in ALOX15B-silenced and ERK1/2-inhibited macrophages, the transcriptional activity of SREBP2 was insensitive to the disruption of intracellular cholesterol transport by inhibition of the lysosomal NPC intracellular cholesterol transporter 1 (NPC1). Taken together, these data provide evidence for a regulatory mechanism based on ALOX15B-mediated lipid peroxidation and concomitant ERK1/2 activation that controls macrophage cholesterol homeostasis.

POSTER 8

ROLE OF SPHINGOSIN-1 PHOSPHATE AND ITS RECEPTORS IN GROUP-B ENTEROVIRUS-RELATED MYOCARDITIS

Eve Vassil, Maxime Bertrand, Domitille Callon, Yohan N?Guyen, Laurent Andreoletti and **Fatma Berri**

Myocarditis is an inflammatory disease of the heart muscle. In majority of cases, this pathology results from common viral infections or post-viral immune mediated response. Human group B enteroviruses (EV-B), specifically Coxsackievirus B3, are infectious common causes of acute myocarditis in children and young adults. Chemical modulators of cellular receptors of the family of Sphingosin-1 Phosphate, play critical roles in immune response and clinical outcome of the several diseases. Sphingosine 1-Phosphate, a natural ligand of S1PR, is a circulating bioactive lipid metabolite. S1P signaling exerts potent cardio-protective and immunomodulatory actions. It was shown that pharmacological activation of S1PR with a common agonist was associated with a protective effect in murine model of myocarditis induced by Encephalomyocarditis virus. However, the impact of EV-B infection on S1P secretion and S1PR expression, and their link with development of myocarditis remain unknown.
In a clinical retrospective study, we evaluated the potential association between S1P concentrations in blood and S1PR expression in heart tissues of patients with EV-B-induced myocarditis. Our results showed that EV-induced myocarditis was associated with significantly higher S1P levels in plasma from patients died from EV-B myocarditis compared to control patients. In cardiac tissue, we observed that EV infection increased mRNA expression of S1PRs, especially S1PR1 and S1PR3. The confocal microscopy analyses show an increase in S1PR3 fluorescence signal in vascular endothelium in the group of patients died from EV-myocarditis. In addition, using a validated mice model for Enterovirus inducing myocarditis, a significant increase in S1P levels in the plasma and mRNA of SphK (1 and 2) in heart tissues were observed at acute stage of CVB3 induced myocarditis in DBA/2J mice. These observations were confirmed in in vitro investigation using a primary human vascular endothelial cell. Interestingly, mice infected with CVB3 and treated with a specific agonist of S1PR1, either at day 0 or at day 3 post-infection, display a significant higher survival rates compared to infected and untreated mice.
Thus, these results suggested a potential implication of the S1P/S1PR system in EV-B-induced myocarditis and point out the possibility of future therapeutic strategies by modulating S1PR activation in viral myocarditis.

POSTER 9

THE SCOTTISH METABOLOMICS NETWORK

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The Scottish metabolomics network was formed in 2015 with a view to bringing together researchers in metabolomics from across the different universities and institutes throughout Scotland. Founded as a way for instrument users to discuss their research and any challenges and solutions associated with running mass spectrometry, and other analytical tools, we have grown to include a large number of PIs whose work is presented at our annual meeting, either by themselves or by group members. This has included work presented by industrial and collaborative partners whose work span the full range of biological small molecule analysis, from breath analysis to bio-fuels, marine biology, human health and nutrition and disease diagnosis and progression monitoring. Along with the presentation of ongoing research, the network offers a way for the people who run the instrumentation to communicate with each other, discuss any issues in a more informal setting and seek out help and advice . This has been supported by us with funding for inter-laboratory exchanges, so researchers are able to visit other labs to learn new methods and techniques.

As a collection of researchers focused on small molecule detection, lipids have been an integral part of our discussions and work, with many groups in the network involved in lipidomics research. This is an area of increasing interest within our network and has seen an increase in the number of presentations at our annual events.

As a network we are continually looking to grow and expand our activities, with plans for regular discussion groups in the works centered around the different areas of the analytical pipeline, from sample prep, chromatography and mass spectrometric methods to data pre-processing and statistical analysis. If you are interested in finding our more about our network then please come and find us at our poster or during the meeting to discuss the network.

POSTER 10

THE LINOLEIC ACID-DERIVED LEUKOTOXIN 9,10-DIHOME DRIVES IMMUNOSUPPRESSION IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

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Introduction: Acutely decompensated (AD) cirrhosis is characterized by smoldering systemic immunosuppression and hyperinflammation that favor the development of secondary infections and multiple organ failure, a condition known as acute-on-chronic liver failure (ACLF) associated with high short-term mortality. In this study, we explored the profile of immunomodulatory lipid mediators in patients with AD cirrhosis with and without ACLF and investigated their effects on leukocyte function.

Materials and methods: The profile of 101 lipid mediators was determined by targeted lipidomics using liquid chromatography coupled to tandem mass spectrometry in plasma from 84 patients with AD cirrhosis without ACLF (stratified as stable decompensated cirrhosis (n = 53), unstable decompensated cirrhosis (n = 10) and patients with high-risk of developing ACLF (pre-ACLF) (n = 21)), and 9 patients with AD cirrhosis who had developed ACLF. For comparison, 31 healthy donors were included. Bioassays determining degranulation, respiratory burst capacity and phagocytosis were performed to assess changes in polymorphonuclear leukocyte function whereas changes in cytokine production capacity, endoplasmic reticulum (ER) stress and mitochondrial dynamics were determined in mononuclear leukocytes by multiplex assays and Western blot analysis.

Results: The exploratory analysis of the baseline lipid mediator profile showed higher plasma levels of linoleic acid (LA)-derived lipid mediators in patients with AD cirrhosis as compared to healthy controls. LA-derived 9,10-dihydroxy-12-octadecenoic acid (9,10-DiHOME) was the only lipid mediator with discriminating power between AD patients with established ACLF from those without. Plasma levels of 9,10-DiHOME followed the severity course of the disease, were significantly higher at the time AD patients manifested an active infection and peaked at ACLF presentation. Moreover, expression of soluble epoxide hydrolase (sEH), the enzyme responsible for the biosynthesis of 9,10-DiHOME, was markedly upregulated in mononuclear leukocytes from AD patients. In the polymorphonuclear leukocyte bioassays, 9,10-DiHOME impaired degranulation, phagocytosis, and respiratory burst capacity, whereas in the mononuclear leukocyte bioassays, 9,10-DiHOME significantly impaired cytokine secretion, induce d ER stress and perturbed the mitochondrial fusion-fission balance.

Conclusion: Leukotoxin 9,10-DiHOME induces immunosuppression suggesting that increased levels of this lipid mediator might enhance susceptibility to bacterial infection and precipitate ACLF in patients with AD cirrhosis. In addition, these findings position sEH as a potential drug target for this condition.

POSTER 11

THE FORMATION OF ENZYMATICALLY-OXIDISED PHOSPHOLIPIDS IN COAGULOPATHIES ASSOCIATED WITH SYSTEMIC INFLAMMATION

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Enzymatically-oxidised phospholipids (eoxPLs), which include hydroxyeicosatetraenoic acid- phosphatidylethanolamines (HETE PEs) generated by lipoxygenases (LOXs) contribute to a prothrombotic surface by supporting PS-dependent binding of coagulation factors. Here, their generation and role were compared in abdominal aortic aneurysm (AAA) and rheumatoid arthritis (RA), using mouse models and human cohorts.

In mice, genetic deletion of platelet 12-LOX or leukocyte 12/15-LOX reduced the development of AAA in a chronic inflammatory model driven by hyperlipidaemia and hypertension (ApoE-/- + AngII infusion).

Furthermore, in wild-type mice, HETE-PLs were detected in AAA lesions and circulating thrombin-antithrombin (TAT) complexes were elevated [1]. In contrast, in a more acute AAA model (elastase + anti- TGFbeta), no role was found for either LOX isoform and coagulation parameters were not elevated. This suggests that blood cell LOXs selectively drive a form of AAA associated with chronic vascular inflammation and hyperlipidaemia.

In the case of RA, antigen-induced arthritis (AIA) was induced in WT, Il27ra-/- and Il6ra-/- mice, which develop histological phenotypes similar to human disease. At day 10, WT and Il27ra-/- AIA mice showed elevated eoxPLs, primarily 12-HETE-PEs in whole blood cells, as well as higher TAT complexes in plasma, while no difference was observed in a low inflammatory phenotype - Il6ra-/-. In addition, the deletion of Alox12, but not of Alox15, prevented the increase of both eoxPLs and TAT complexes in the blood of AIA mice, suggesting platelet 12-LOX was contributing to increased coagulation.

In human AAA, several eoxPLs were detected in both vessel wall and associated thrombus [1]. In a human RA cohort, platelet counts were elevated. Furthermore, an increase in plasma IgG immunological recognising eoxPLs was found, suggesting higher circulating levels of eoxPLs occur in vivo.

In summary, eoxPLs from LOX enzymes may play a functional role in the elevated thrombotic risk in inflammatory conditions, including AA and RA.

[1] K. Allen-Redpath et al., "Phospholipid membranes drive abdominal aortic aneurysm development through stimulating coagulation factor activity," Proc Natl Acad Sci U S A, vol. 116, no. 16, pp. 8038–8047, 2019, doi: 10.1073/pnas.1814409116.

POSTER 12

THE ROLE OF THE INTESTINAL MICROBIOME IN THE REGULATION OF PRO-RESOLVING LIPID MEDIATORS

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Bsc Thesis Abstract

BACKGROUND: Chronic inflammation is being identified as a mutual driver of various non-communicable diseases. The lipid mediators involved in initiating and resolving inflammation are synthesised from polyunsaturated fatty acids (PUFA) through multi-step processes, which are influenced by several endogenous and environmental factors. Among these environmental factors, dietary habits also shape the composition and function of the intestinal microbiome. Since more than one-third of serum metabolites are solely associated with the intestinal microbiome, they may play a key role in regulating lipid mediator biosynthesis and the resolution of inflammation.

METHODS: This thesis aims to be the first published review to synthesise the available literature on the host-microbiome interactions that regulate lipid mediators and draw nutritional conclusions. Based on robust long-term research data and the Dietary Inflammatory Index (DII), omega-3 PUFAs and short-chain fatty acids (SCFAs) were chosen as candidate compound groups to evaluate their molecular interactions in inflammation resolution.

RESULTS: The findings indicate that omega-3 PUFAs act as prebiotics, promoting SCFA production from dietary fibre by the beneficial intestinal microbiota species. Additionally, some bacteria can even convert dietary PUFA to pro-resolving oxylipins, thus producing invaluable postbiotics intraluminally. Moreover, multiple preclinical studies confirm the hypothesised synergistic interactions between SCFAs and omega-3 PUFAs in regulating the biosynthesis and signaling of pro-resolving lipid mediators.

CONCLUSIONS: Based on the molecular interactions, SCFAs, especially butyrate, promote the signal transduction of inflammation resolution through multiple direct and indirect pathways. The nutritional implications highlight the outstanding potential of this synergy for future translational studies investigating chronic inflammation-associated diseases.

POSTER 13

TOTAL SYNTHESIS AND ANTI-INFLAMMATORY ACTIONS OF RESOLVIN D5N-3 DPA

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Resolvin D5n-3 DPA, a specialized pro-resolving mediator derived from the omega-3 polyunsaturated fatty acid n-3 docosapentaenoic acid (n-3 DPA), was reported in 2013. The lipid exhibits anti-inflammatory properties, hence serving as an interesting lead for drug discovery based on resolution pharmacology. In this poster, the stereoselective total synthesis and biological evaluation of the mediator will be presented.

POSTER 14

INVESTIGATING FORMYL-PEPTIDE RECEPTOR 1 (FPR1) SIGNALLING AND OTHER LIPID MEDIATOR PATHWAYS FOR MODULATING NEUTROPHIL ACTIVITY IN INFLAMMATION

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Neutrophil activation is induced by mediators such as the arachidonic acid metabolite lipid LTB_4, the ether lipid PAF, and peptides such as fMLF and IL-8. All these agonists trigger a similar series of neutrophil functions including chemotaxis, reactive oxygen species (ROS) production and degranulation which results, if dysregulated, in an enhanced inflammatory response and subsequent tissue damage in numerous neutrophil dominant diseases, partly due to endogenous release of neutrophil derived LTB_4 and PAF. Therefore, in this study we aim to use existing and novel pharmacological modulators/inhibitors (ADS051), to target these key lipid mediator pathways to modulate human neutrophil responses. ADS051 is a novel, oral, gut-restricted, small molecule. ADS051 modulates neutrophil trafficking and activity via the *MRP2 (*multidrug resistance protein 2) and *FPR1 (*formyl peptide receptor 1) mediated mechanisms in human cell-based systems. Its lack of both systemic exposure and blockage of T cell activation is intended to limit immunosuppression.

Neutrophils isolated from healthy human blood by dextran sedimentation, followed by Percoll discontinuous gradient centrifugation were pre-incubated with fMLF (targeting FPR1), LTB_4 (for the BLT1 receptor) and PAF (for the PAFR) in the absence and presence of modulators/antagonists [e.g., cyclosporin-H (CsH) and ADS051 an FPR1 antagonist and modulator, respectively) and CP-105696 (a BLT1 receptor antagonist], prior to agonist stimulation. Shape-change (polarisation) in human neutrophils was measured as alterations in forward-scatter and changes in adhesion molecule (CD62L and CD11b) expression were analyzed by flow-cytometry, to characterize neutrophil activation. Intracellular Ca^{2+}-mobilization was determined using Fluo-4AM fluorescence using high-content confocal microscopy and the production of intracellular ROS using the cell permeable fluorogenic probe, 1,2,3-dihydrorhodamine (DHR) using flow cytometry.

LTB_4, PAF, fMLF and IL-8 resulted in concentration-dependent polarisation of human neutrophils, which was rapid and reversible. CsH (20μM) inhibited fMLF-induced alterations in neutrophil shape change, adhesion molecule expression, intracellular Ca^{2+}mobilization and ROS production in human neutrophils. ADS051 also inhibited neutrophil polarisation. CsH did not attenuate LTB_4 (100nM), PAF (100nM) or IL-8 (50ng/mL)-mediated neutrophil polarisation (shape-change) suggesting specific effect on the FPR1 signalling pathway. Conversely, CP-105696 (1μM) significantly inhibited LTB_4 mediated human neutrophil polarisation but failed to antagonize FPR1.

Our data with CP-105696 further suggests that the release of LTB_4 and other lipid mediators do not contribute to the above neutrophil responses caused by other agonists. These observations suggest that LTB_4, PAF, fMLF and IL-8 are independent human neutrophil activators and both CsH and ADS051 are antagonists or modulators of the FPR1 signalling pathway. The novel gut-restricted design of ADS051 allows for oral dosing and neutrophil modulation locally, at the site of bowel inflammation.

POSTER 15

OBESITY ASSOCIATED INFLAMMATION IN ADIPOSE TISSUE IS CONTRIBUTED TO BY DEPLETION OF DHA CONTAINING MEDIATORS AND TRANSCRIPTOMIC DYSREGULATION WHICH MAY LINK LIMITED RESPONSES TO LC N-3 PUFA INTERVENTION.

Helena Fisk, Rob Ayres, Caroline Childs, Paul Noakes, Ondrej Kuda3, Jan Kopecky, Elie Antoun, Karen Lillycrop, Anandita Pal, Saame Raza Shaikh, Philip Calder

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Obesity is an excess of adipose tissue (AT) and is linked with increased inflammation that enhances risk of type-2 diabetes and cardiovascular disease. The BIOCLAIMS Study assessed AT inflammation in obesity, and responses to chronic omega-3 fatty acid (FA) supplementation.

AT biopsies and blood were collected pre- and post-12-week supplementation with 1.1g EPA + 0.8g DHA/day or corn oil. AT FA composition, AT and blood lipid mediator profile, AT whole transcriptome expression, morphology and immune cell infiltration were assessed by gas chromatography, coupled UPLC-mass spectrometry, RNA-Sequencing, and immuno-histochemical staining respectively.
Obesity was associated with dysregulated FA and lipid mediator profiles exhibiting higher concentrations of arachidonic acid (AA) and respective oxylipins, lower concentrations of DHA oxylipins, and alteration of the endocannabinoid system (ECS) ($P < 0.05$). Depletion of DHA oxylipins was not reflected in blood profiles. In addition, obesity was associated with an altered transcriptome expression suggestive of enhanced inflammation, immune response, and tissue expansion. Altered lipid mediator profiles may be linked to transcriptional changes to lipid handling; 5-LOX and COX-1 were upregulated and SLC27A2 encoding very long chain acyl co-A synthetase, required for the activation and transport of long chain FAs was downregulated by 92% in obesity ($P <0.05$). Tissue expansion and inflammation was concordant with tissue morphology in which obesity was associated with adipocyte hypertrophy and immune cell infiltration ($P < 0.05$).
Chronic supplementation with EPA+DHA increased concentrations of AT omega-3 FAs ($P < 0.01$) in both groups but only increased omega-3 derived oxylipins in healthy-weight individuals. EPA+DHA supplementation decreased AA oxylipins, particularly modulating the ECS in both groups ($P < 0.05$), and modulated AT transcriptome suggesting promotion of tissue remodelling and downregulation of chronic inflammatory response ($P < 0.05$).

In summary, obesity is associated with enhanced inflammation and changes to transcriptional regulation of lipid metabolism which may link to depleted DHA oxylipins and limited responses to EPA+DHA intervention. The role of SLC27A2 oxylipin synthesis and its affinity for DHA in AT is not known and further investigation is required. Overcoming these differences to restore lipid metabolism and oxylipin signalling may be a route to resolving inflammation in obesity.

POSTER 16

BIOACTIVE LIPID MEDIATORS IN SUBARACHNOID HAEMORRHAGE

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Background
This study addresses the critical role of bioactive lipid mediators derived from polyunsaturated fatty acids (PUFAs) in subarachnoid hemorrhage (SAH). Using liquid chromatography-tandem mass spectrometry (LC- MS/MS), we aim to identify and quantify such mediators in SAH patients and healthy controls at admission, monitor their changes over time, and assess their potential in predicting neurological outcomes, including delayed cerebral ischemia (DCI), a severe complication characterized by reduced brain blood flow and worsening neurological deficits. Literature highlights the significant influence of bioactive lipid mediators on inflammation and vasoregulation post-SAH. Our findings could therefore provide insights into the pathophysiology of SAH, identifying potential biomarkers for prognosis and therapeutic targets, thus improving patient management and outcomes.

Aim - To comprehensively characterize lipid mediator profile in SAH patients, aiming to enhance insights into underlying pathological mechanisms. We will investigate whether lipid mediator profiles can be used as biomarker to differentiate between SAH patients with and without delayed cerebral ischemia (DCI), potentially serving as predictive markers for adverse outcomes.

Methods - We utilized LC-MS/MS to establish a comprehensive lipid profile from plasma samples of subarachnoid hemorrhage (SAH) patients, as well as patients with unruptured aneurysms (UA) and healthy controls (HC). Peripheral blood was collected upon admission and at days 4, 10, and 21 for SAH patients, while only one time point was included for the UA and HC groups. SAH patients were further categorized into two age- and sex- matched groups based on the development of delayed cerebral ischemia (DCI) within two weeks.

Results/Conclusions - Our study comprised 52 SAH patients, including 26 with delayed cerebral ischemia (DCI), alongside 26 unruptured aneurysm (UA) patients and 13 healthy controls (HC). We observed elevated levels of 11-, 12-, 15-, and 20-HETE in all SAH patients compared to the UA group upon admission. Furthermore, SAH patients exhibited significant changes in lipid profiles over time. Interestingly, only 8-HETE demonstrated possible potential as a differentiator between DCI and non-DCI patients at admission. However, despite these findings, the lipid mediator profile upon admission did not prove predictive of DCI development.

POSTER 17

MOTION-BASED DRUG DISCOVERY OF HUMAN 5-LIPOXYGENASE

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Upon cellular stimulation, Human 5-Lipoxygenase (5-LOX) initiates the synthesis of the pro-inflammatory leukotrienes. Calcium, adenosine triphosphate, and phospholipids were some of the earliest discovered activators of 5-LOX and in their absence modest activity is observed. The first crystal structure of a stabilized version of 5-LOX revealed the active-site iron occluded from the bulk solvent, plugged by aromatic residues, which we hypothesize is the inactivate, resting-state structure of protein in the cytosol and and the conformation of the protein encountered during inhibitor incubations. Recently, we solved an “opened'' structure of 5-LOX illustrating the conformational change necessary for formation of the substrate-access portal. We designed new variants of 5-LOX that favor the open conformation by removing the molecular contacts that stabilize the closed structure. A subset of variants shows at least a 10-fold increase in catalytic efficiency. Moreover, single-particle analysis by cryo-EM of these more active variants of 5-LOX revealed the macromolecule accessing highly-populated, “opened” conformations. Additionally, X-ray crystallographic co-structures of 5-LOX with numerous competitive inhibitors have been solved but with little success in defining the electron density of the inhibitor and encapsulating peptides of the active site. These co-structures, with moving peptides around the active site of 5-LOX, recapitulate the movement observed by cryo-EM. We have shifted our focus to a fragment-based approach with the aim of resolving the electron density of the inhibitor and active-site for structure-based drug design. We have been successful at capturing two co-crystal structures of 5-LOX with fragments bound next to the catalytic iron with the aromatic-plugging residues positioned in the closed conformation of the substrate-access portal and sealing the fragment from the bulk solvent. We hypothesize that a conformational change of helix alpha-2 where the plugging residues reside must occur for binding to occur. We propose that conformational movement near the active site of 5-LOX must be considered for inhibitor design (motion-based drug discovery).

POSTER 18

LIPIDOMIC ANALYSIS SUGGESTS MODIFICATION OF LIPID RAFT COMPOSITION VIA SGMS1 AND ELOVL1 IN INFANT LEUKAEMIA CELLS

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Altered lipid metabolism is a well-recognised phenomenon in many types of cancers, but poorly understood in infant leukaemia. Our group identified two potential regulators of t(4;11) MLL-AF4+ acute lymphoblastic leukaemia (ALL) related to lipid metabolism, SGMS1 (sphingomyelin synthase 1) and ELOVL1 (very long-chain fatty acid elongase1). We previously observed SGMS1 downregulation and ELOVL1 upregulation in MLL-AF4+ ALL infant patients, murine models and human leukaemic cell lines. Functional experiments (SGMS1 overexpressed, ELOVL1 knockout/inhibited) showed these regulators play an important role, as tumour suppressor and as oncogene respectively. Therefore, we aimed to understand how lipid composition in MLL-AF4+ leukaemia was modified by SGMS1 and Elovl1.

We analysed lipidomic datasets from (1) SEM cells (paediatric MLL-AF4+ ALL cell line) with/without SGMS1 overexpression, (2) SEM cells +/-bezafibrate (Elovl1 inhibitor), (3) Per494 cells (infant MLL-AF4+ ALL cell line) +/- bezafibrate, (4) E14.5 control foetal liver haematopoietic stem and progenitor cells and leukaemic cells from mouse models of infant ALL (5) and mixed-phenotype acute leukaemia (6) developed in our lab.

SEM cells overexpressing SGMS1 showed reduced levels of cholesterol esters (CE) and triglycerides (TG) compared with control cells. These latter were rich in polyunsaturated fatty acids (PUFA) containing â‰¥5 unsaturations.
Cells (SEM and Per494) treated with bezafibrate showed low levels of CE, TG, and diacylglycerides, and high levels of phosphatidylinositol and sphingomyelin (SM) compared to controls. Such pattern was also found in our ALL murine model, but not in our mixed-phenotype murine model, suggesting that this lipid composition is ALL specific. PUFA storage in untreated leukaemic cells could be linked to higher resistance to ROS, a known phenomenon in cancer cell resistance to treatments. CE and SM are main components of lipid rafts, and modulation of raft composition is related to resistance to apoptosis and chemotherapy in several types of cancer. Lipid Ontology enrichment analysis showed enriched terms related to lipid rafts properties. These preliminary data suggest that lipid modulation in MLL-AF4+ leukaemia may be important for leukaemogenesis and treatment resistance, being an unexplored vulnerability of infant leukaemia cells. Future functional investigations will involve raft formation, raft-associated protein identification and their modulation by SGMS1/ELOVL1.

POSTER 19

SYNTHESIS AND BIOLOGICAL EVALUATION OF 14,15-EPOXYEICOSATRIENOIC ACID AND ITS DERIVATIVES

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Epoxyeicosatrienoic acids (EETs) play a crucial role in the metabolism of arachidonic acid. Their formation in vivo is due to the epoxygenase activity of the cytochrome P450 system. These enzymes, mainly members of the CYP P450 2C and 2J subfamilies, can produce four different EETs starting from arachidonic acid (5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET). EETs act as autocrine and paracrine effectors but their exact physiological role remains unknown. Previous research describes their involvement in different signaling pathways. As a result, EETs have pleiotropic effects including anti-inflammatory, anti-thrombotic, vasodilatory and proangiogenic activities. EETs also play a role in the regulation of vascular homeostasis and have protective effects on the cardiovascular system. All four EETs are rapidly hydrolyzed to the less beneficial dihydroxyeicosatrienoic acids (DHETs) by the soluble epoxide hydrolase (sEH).[1,2]
Out of the four EETs, 14,15-EET is a favored product of the CYP-catalyzed epoxidation reaction and can be selectively and efficiently synthesized from arachidonic acid.[3] We used this as a starting point for the synthesis of several 14,15-EET derivatives and are aiming to explore their physiological functions. The compounds we synthesized are conjugates of 14,15-EET with different functional groups that may be present in vivo. These include the epoxidized derivatives of arachidonoyl monoacylglycerol (MAG), lysophosphatidic acid (LPA), anandamide and the paracetamol metabolite N-arachidonoylphenolamine (AM404).
Our study focuses on the synthesis of these compounds as well as their biochemical screening in order to investigate their molecular mode of action. We could show that 14,15-EET-2-monoacylglycerol (14,15-EET-2- AG) is a potent agonist on the cannabinoid receptor 1. It has also previously been reported that EETs can activate PPAR gamma and thus act as anti-inflammatory agents.[4] We are currently working on exploring the receptor activation of these compounds, especially related to GPCRs and nuclear receptors.

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POSTER 20

A 3D NEUROSTEROIDS ATLAS OF MOUSE BRIAN USING MASS SPECTROMETRY IMAGING

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Neurosteroids are synthesized locally within the central nervous system and play essential roles in modulating neuronal activity and various brain functions. They can have a wide range of effects, including anxiolytic, sedative, analgesic, and neuroprotective actions. Understanding their distribution in the brain and how they interact with neurotransmitter systems is of significant interest to researchers studying brain function and related disorders.

We aim to construct a 3D atlas of a panel of neurosteroids to uncover the complex network of functions of neurosteroids in the mouse brain. Mass spectrometry imaging (MSI) is a powerful bioimaging tool that combines mass spectrometry with spatial information to three-dimensional maps of the distribution of molecules within a sample with direct histopathological correlation.

Matrix assisted laser desorption ionisation (MALDI)-MSI was used to create distribution maps of neurosteroids, with 100 and 150 µm spatial resolution, from brains of 56-days-old male and female C57BL/6 mice. Serial sagittal 10µm cryostat brain sections were collected at around 200 µm intervals across the right hemisphere from cortex to midline. On-tissue chemical derivatisation with Girard-T reagent was applied to enhance the signal sensitivity of detection of neurosteroids containing keto functional groups. MSI data were collected on Bruker-12T-SolariX-Fourier-transform–ion-cyclotron-resonance (FT-ICR)-MS. Estrone, androstenedione, 7aOH-DHEA, progesterone, 17aOH-Progesterone/11-deoxycorticosterone, 11-dehydrocorticosterone and corticosterone were detected in brains from both sexes, mainly localised in cortex, hippocampus and cerebellums. Z-Stacking of sequential MSI plates allow generation of 3D models.

Future work includes MSI data alignment and co-registration with the 20 Allen Mouse Brain Reference Atlas. This will be made publicly available via interactive webpages to allow precise anatomical annotations to search and visualise concentrations of individual neurosteroids in different areas of the mouse brain.

POSTER 21

HOW AN EX-VIVO N-3 PUFA SUPPLEMENTATION OF MACROPHAGES CAN MIMIC HUMAN NUTRITION STUDIES

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Increased consumption of long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) is linked to positive health outcomes and anti-inflammatory effects. n-3 PUFA undergo both enzymatic and non-enzymatic oxidation, resulting in a variety of different oxylipins. Several of these oxylipins are potent bioactive lipid mediators playing an important role in the regulation of physiological processes such as pain and inflammation. Thus, oxylipins are believed to be part of the mode of action by which n-3 PUFA exert their anti-inflammatory properties. However, the underlying modes of action are still subject of ongoing research.

For a detailed investigation of the effects of n-3 PUFA and arising oxylipins in human immune cells, an n-3 PUFA ex-vivo supplementation strategy was developed. Primary human macrophages derived from blood monocytes were supplemented with different concentrations of n-3 PUFA for different periods of time. For investigation of potential anti-inflammatory effects of the n-3 PUFA, macrophages were stimulated with bacterial lipopolysaccharide (LPS) and analyzed for changes in the oxylipin, protein and mRNA levels.

After optimization of the experimental strategy, a reliable and reproducible supplementation with n-3 PUFA was achieved: We show, how the cellular FA pattern of monocytes derived from human subjects following a typical Western diet was changed to macrophages having a FA pattern associated with a high n-3 PUFA status.

Here, we present how the n-3 PUFA supplementation changes the oxylipin pattern, protein abundance and mRNA levels with and without LPS stimulation. Overall, the developed strategy is a promising tool for further mechanistic investigation of n-3 PUFA effects under tightly controlled conditions without the need for intervention studies in humans.

POSTER 22

EFFEROCYTE-DERIVED MCTRS PRIME MACROPHAGES FOR CONTINUAL EFFEROCYTOSIS VIA RAC1-MEDIATED ACTIVATION OF GLYCOLYSIS

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Clearance of multiple rounds of apoptotic cells (ACs) by macrophages through continual efferocytosis is critical for the maintenance of organ function, resolution of acute inflammation, and tissue repair. We therefore set out to identify mechanisms and factors that govern this fundamental process. We found that ingestion of ACs led to the upregulation of 12S-lipoxygenase (ALOX12) expression in human macrophages. This upregulation was dependent on recognition of AC-derived DNA by toll-like receptor 9 and activation of the aryl hydrocarbon receptor. Overexpression of 12S-lipoxygenase in human macrophages led to increased AC clearance by both the overexpressing cells themselves as well as nearby macrophages, while mouse macrophages lacking the orthologous 12/15-lipoxygenase (Alox15) showed reduced efferocytosis that was restored upon co-culture with 12/15-lipoxygenase-expressing macrophages.

12S-lipoxygenase is involved in the biosynthesis of the efferocytosis-promoting specialized pro-resolving mediators termed maresins and maresin conjugates in tissue regeneration (MCTRs) from docosahexaenoic acid. We found that MCTR levels were elevated at sites of high AC burden in vivo and in efferocytosing macrophages in vitro. On the other hand, macrophages from 12/15-lipoxygenase-deficient mice showed defective continual efferocytosis both in vivo and in vitro, an effect that was rescued by add-back of MCTRs.

Mechanistically, MCTR-mediated priming of macrophages for continual efferocytosis was dependent on alterations in Rac1 signalling and glycolytic metabolism. Inhibition of Rac1 abrogated the ability of MCTRs to increase glucose uptake and efferocytosis in vitro, whereas inhibition of glycolysis limited the MCTR-mediated increases in efferocytosis. Taken together, our findings demonstrate that upregulation of MCTRs by efferocytosing macrophages plays a central role in continual efferocytosis via the autocrine and paracrine modulation of key metabolic pathways that facilitate AC uptake by macrophages.

POSTER 23

SYNTHESIS AND CHARACTERIZATION OF NEW OCTADECANOID STANDARDS

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Oxylipins are metabolites produced by the oxidation of polyunsaturated fatty acids (PUFAs). They have been extensively studied for their roles as mediators of inflammation and their involvement in a wide variety of diseases. While most of the attention has been dedicated to oxylipins derived from 20-carbon PUFAs, collectively known as eicosanoids and which include key mediators such as prostaglandins and leukotrienes, there is increasing interest in oxylipins derived from other PUFAs. The oxidative enzymatic pathways of octadecanoids, particularly linoleic acid (FA18:2 n-6) and α-linolenic acid, have been shown to be similar to those of eicosanoids, including cyclooxygenases, lipoxygenases, and cytochrome P450. However, standards for their unequivocal identification and quantitation are not always available. This study shows the synthesis of twelve oxylipins derived from two common 18-carbon fatty acids with three double bonds, ALA (α-linolenic acid; FA18:3n-3), and GLA (γ-linolenic acid; FA18:3n-6), both of which are widely distributed in many living organisms. The standards synthesized are all epoxides and diols derived from these two fatty acids: α-9(10)-EpODE (α-9,10-epoxy-octadecadienoic acid); α-12(13)-EpODE; α-15(16)-EpODE, γ-6(7)-EpODE; γ-9(10)-EpODE; γ-12(13)-EpODE; α-9(10)-DiHODE (+/-9,10-dihydroxy-octadecadienoic acid); α-12(13)-DiHODE; α-15(16)-DiHODE; γ-6(7)-DiHODE, γ-9(10)-DiHODE; and γ-12(13)-DiHODE. These standards were synthesized with purities of at least 95%, as determined by HPLC. The data presented show the unequivocal characterization of these standards by their tandem mass spectrometry (MS/MS) spectra, and, for the six epoxides, also by their 1H NMR spectra. These new standards will make it possible to identify and quantify these analytes in biological sample, as well as monitor the in vivo activities of enzymes such as soluble epoxide hydrolase (sEH). This will help investigate the roles of these octadecanoids in disease, and to assess their potential as targets of pharmaceutical treatment, expanding the availability of oxylipin standards available to the scientific community, which is critical to advancing research of this biochemical space.

POSTER 24

EFFECTS OF INTERMITTENT FASTING ON BLOOD FATTY ACIDS AND OXYLIPINS

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Background: Intermittent fasting (IF) is a dietary form of energy restriction that is receiving increasing attention for reducing metabolic risk and inhibiting inflammatory processes. Furthermore, calorie-restriction and fasting diet interventions have been shown to increase lifespan and healthspan in experimental contexts. Although there is increasing data on the positive effects of intermittent fasting on metabolic health, there is still uncertainty about underlying mechanisms. Objective: To investigate the short-term effects of IMF on metabolic parameters and blood fatty acids levels with special regard to omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) important for metabolic and inflammation regulation.

Methods: Patients with NAFLD or dyslipidaemia as well as healthy subjects were recruited to at the metabolic outpatient clinic of Brandenburg Medical School after they received dietary advice and decided to follow a 16:8 hour IF diet approach. Patients were followed at baseline and follow-up after 4- and 8-weeks, anthropometric data were recorded and lipidological parameters and plasma fatty acids as well as oxylipins were determined via gas chromatography. Additionally, FibroScan elastography was used to quantify liver fat and the degree of liver stiffness.

Results: At follow-up, body weight and BMI decreased significantly in response to IF. Among fatty acids, the omega-6 PUFA arachidonic acid (AA) increased significantly, without increased AA-derived oxylipins.

POSTER 25

STUDY OF THE EFFECT OF MAGL INHIBITION IN ZYMOSAN-INDUCED PERITONITIS

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The endocannabinoid system plays well described roles in many physiological and pathological processes. Contemporary research increasingly focuses on its involvement in inflammatory responses, yet its role in the resolution of inflammation remains unexplored. The resolution of inflammation constitutes a complex mechanism that has garnered significant interest, particularly as it relates to developing therapeutic strategies for chronic inflammatory conditions. The persistence of unresolved inflammation is known to contribute to the etiology of numerous diseases. Furthermore, while current anti-inflammatory treatments prove efficacious in managing acute inflammation, their long-term effectiveness diminishes.

In this context, we used a widely recognized model for discerning various pro-resolutive agents, namely the zymosan-induced peritonitis. Our investigations centered on the expression profiles of several enzymes critical to the metabolism of endocannabinoids within this model. Of particular interest was monoacylglycerol lipase (MAGL), which exhibited increased expression at the peak of inflammation, while its substrate, the endocannabinoid 2-arachidonoylglycerol (2-AG) showed elevated levels during the resolution phase. Given these observations and the known beneficial effects of 2-AG on inflammation, we sought to examine the implications of MAGL inhibition on the inflammatory resolution process.

The administration of a selective MAGL inhibitor resulted in increased 2-AG levels and showed interesting properties in the context of zymosan-induced peritonitis. Indeed, we found a decreased number of infiltrating neutrophils, an improvement of resolution parameters and zymosan clearance while augmenting neutrophils efferocytosis and, consequently, shortened the duration of the inflammation.
These encouraging findings pave the way for further investigations into the endocannabinoid system's role in resolving inflammation, offering promising possibilities for future target identification.

POSTER 26

EXPRESSION OF ENZYMES INVOLVED IN LONG-CHAIN FATTY ACID PROVISION UPSTREAM OF OXYLIPIN FORMATION IN HUMAN LEUKOCYTES

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Pro-inflammatory lipid mediators such as prostaglandins (PG) and leukotrienes (LT) as well as pro- resolving lipoxins and dihydroxylated resolvins are produced by human leukocytes during the time course of an inflammation. While PGs and LTs initiate and maintain the inflammatory process, lipoxins and resolvins are formed towards the end of the inflammation to actively orchestrate its resolution.

The central biosynthesis of these oxylipins has been well researched: Through inflammatory stimulation of macrophages and granulocytes, polyunsaturated fatty acids (PUFA) such as arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are released from phospholipids of the cell membrane by phospholipases and are then available to the cyclooxygenase (COX) or lipoxygenase (LO) pathway. In the former, ARA is converted into a range of different PGs, depending on the cell type. In the latter pathway, ARA, EPA or DHA are converted into LTs, lipoxins and resolvins by one or more LOs (5-, 12-, or 15- LO). Prior to the release of the oxylipin precursors (ARA, EPA, DHA, monohydroxylated derivatives), the uptake and membrane storage of these precursors in the synthesizing leukocytes is of central importance in lipid mediator formation, especially in transcellular biosynthesis. However, little is known about these processes.

In the present study, we investigated the expression of proteins involved in transport, activation and phospholipid incorporation of long-chain fatty acids in human primary leukocytes and in the myeloid cell lines THP-1, MonoMac-6 and HL-60.

POSTER 27

PLASMA PATTERNS OF N-6 AND N-3 PUFA OXYLIPINS IN PATIENTS WITH DIFFERENT GENOTYPES FOR TM6SF2 AND PNPLA3

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TM6SF2 (transmembrane 6 superfamily member 2) and PNPLA3 (Patatin-like phospholipase domain-containing protein 3, adiponutrin) are proteins that play important roles in the regulation of liver function and fatty acid metabolism. Variants of both were shown to increase susceptibility to fatty liver disease. In this study we assessed these genotypes in the context of polyunsaturated fatty acid and oxylipin levels in blood samples from 110 patients from our metabolic clinic. Patients were characterized for their liver fat content, routine lipid blood parameters, medical history and clinical characteristics as well as genotype of PNPLA3 and TM6SF2 by characterization of specific SNPs.
Profiles of PUFAs and their oxylipins in plasma were measured by gas chromatography and liquid chromatography/tandem mass spectrometry. Our pilot data indicate that the genotype of TM6SF2, but not of PNPLA3, might affect oxylipin plasma levels in metabolic patients with higher plasma levels of CYP- and LOX- oxylipins in carriers of the T-Allele at rs58542926.

POSTER 28

THE OXYSTEROL 4BETA-HYDROXYCHOLESTEROL HAS PRO-HOMEOSTATIC EFFECTS IN THE OB/OB MICE

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Oxysterols are endogenous derivatives of cholesterol whose levels are altered in several pathophysiological situations, most notably in obesity. Our group has shown in several models of obesity, including ob/ob mice, that 4beta-hydroxycholesterol (4beta-OHC) levels are decreased in the serum and in the adipose tissue of obese mice. Interestingly, it was also reported that circulating levels of 4beta-OHC are decreased in obese subjects. As 4beta-OHC has been described to be a Liver X receptor (LXR) agonist and as this receptor is involved in lipid homeostasis and inflammatory response, we hypothesized that 4beta-OHC may modulate the pathophysiology of obesity.

In this work, we report the anti-inflammatory properties of 4beta-OHC on subcutaneous adipose tissue explants from ob/ob mice. Indeed, incubation of the adipose tissue explants with 4beta-OHC reduced the lipopolysaccharide-induced cytokine secretion from the adipose tissue. We also explore the effect of 4beta-OHC administration to ob/ob mice (and their lean littermates) on the inflammation, adipose tissue differentiation and metabolic disorders linked to obesity. While we did not find decreased adipose tissue inflammation following 4beta-OHC administration to ob/ob mice, we found that 4beta-OHC administration increased the small adipocyte population in subcutaneous adipose tissue compared to the ob/ob mice that received the vehicle. This observation led us to study the effects of 4beta-OHC incubation on preadipocyte differentiation using the 3T3-L1 cell line.

Finally, we found that 4beta-OHC administration had some pro-homeostatic effects on metabolism in ob/ob mice (e.g. decreasing glycaemia). These effects were associated to a decreased expression of several metabolic markers known to be increased during obesity. For instance, 4beta-OHC administration reduced the expression of glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), and the transcription factor FOXO1 that were increased in ob/ob mice compared to the lean littermates.

Taken together, these results suggest the involvement of 4beta-OHC in obesity and supports a potential clinical relevance for these bioactive lipids.

POSTER 29

Effect of metformin and aicar on the muscular reactivity of human bronchi and pulmonary artery: role of autophagy regulation and COX-2/PGE2 pathway

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Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by airway inflammation, airflow obstruction and emphysema1,2. Autophagy is a physiological process clearing the senescent organelles to maintain cellular homeostasis3 and autophagy dissonance is obvious in COPD patients4. Metformin is the first-line drug for type 2 diabetes, with aicar they are classical AMPK activators and they can promote autophagy. COX-2/PGE2 pathway is a critical inflammatory pathway in the human lung: PGE2 induces bronchodilatation and vasoconstriction of pulmonary artery (PA), via EP3 and EP4 receptors, respectively. Furthermore, some studies demonstrated that autophagy has a closed relationship with COX- 2/PGE2 pathway5,6. Therefore, the aim of this study is to investigate the effects of metformin and aicar on the reactivity of COPD bronchial and pulmonary artery (PA) preparations setup in an organ-bath system, and on the COX2/PGE2 expressions.

Results: Metformin increased the contraction induced by norepinephrine (NE) in the COPD PA, but aicar slightly reduced contraction; however, metformin and aicar reduced the contraction of non-COPD PA7. On the other hand, metformin slightly reduced the contraction induced by NE of COPD bronchi, in contrast, aicar potentiated bronchoconstrictions. We analysed the expression of COX-2, mPGES1, PGE2 and its EP1-4 receptors in human bronchi homogenates using western blot, RT-PCR, ELISA and immunohistochemistry (IHC). COX-2 and mPGES1 enzymes were significantly increased in COPD patients compared with controls. PGE2 level was significantly increased in COPD patients versus control. EP4 receptor (protein and mRNA) expressions were significantly reduced in the COPD group versus controls and confirmed by IHC. No difference was found with EP1-3 receptor expressions.

Discussion: The effects of metformin and aicar on PA and bronchi reactivity are opposite, which may result from the distinct expressions and activations of EP3 or EP4 receptors in these tissues. Another explanation could be the different mechanisms of autophagy regulation by these two drugs. Although both of them could induce autophagy by activating AMPK, high concentrations of aicar have also been reported to inhibit autophagy8.

Conclusion: Metformin and aicar could regulate differently the reactivity of artery and bronchi, the expression and the underlying mechanism may involve autophagy, COX2 pathway and/or different PGE2 receptors.

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POSTER 30

COMBINED LIPOPROTEIN AND LIPIDOMIC PROFILE PREDICT RESPONSE TO ABATACEPT IN EARLY UNTREATED RHEUMATOID ARTHRITIS

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Biological disease-modifying antirheumatic drugs (bDMARDs), such as abatacept, have demonstrated superior efficiency in rheumatoid arthritis (RA) compared to conventional synthetic disease-modifying antirheumatic drugs (csDMARDs). Despite this, a significant proportion of patients remains unresponsive to bDMARDs, with the absence of reliable biomarkers to predict treatment outcomes. Lipidomic profiles have been shown to be altered in RA and associated with disease severity, but very little is known regarding the prognostic values of lipids in predicting response to bDMARDs in RA. We analysed plasma lipoprofile and lipidome in patients with early untreated RA (euRA) enrolled in the NORD-STAR trial (registration number: NCT01491815) using combined nuclear magnetic resonance (NMR)-differential mobility spectrometry-mass spectrometry (DMS-MS) approaches. Participants included in the NORD-STAR trial were randomized into four therapeutic regimes: methotrexate (MTX) combined with either prednisolone, certolizumab pegol, abatacept, or tocilizumab. A subgroup of patients, identified by elevated baseline levels of very-low-density lipoprotein (VLDL) subfractions and triglyceride (TG) species, showed a poor response to abatacept. Abatacept non-responders, defined as clinical disease activity index (CDAI)ï¼ž2.8 at week 12, had significantly higher levels of ceramides and TG with very-long-chain polyunsaturated fatty acid (TG-VLC-PUFA) species at baseline. Overall, our research underscores the utility of plasma lipidomic profiling as a predictive biomarker for abatacept responsiveness in euRA, offering a promising avenue for personalized treatment strategies.

POSTER 31

BEYOND 9- AND 13-H(P)ODE: EXPLORING NEW OXYLIPINS IN EDIBLE OILS

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Polyunsaturated fatty acids (PUFA), particularly linoleic acid (C18:2n6, LA) and a-linolenic acids (18:3n3, ALA) are found in high amounts in edible oils. Both enzymatic and non-enzymatic oxidation can lead to the formation of a large number of oxylipins. Primary oxidation products are hydroperoxy-PUFA, which can be reduced to hydroxy-PUFA or undergo further reactions.
Despite the profound knowledge about oxylipin formation in plants and mammals, information about the oxylipin pattern in food is sparse. Using a comprehensive non-targeted liquid chromatography high resolution mass spectrometry (LC-HRMS) analysis we could show, that many previously unknown oxylipins occur in edible oils. Indeed, some of them occur in higher concentrations than well described oxylipins such as 9-HODEand 13-HODE.

In flaxseed oil, three 12-hydroxy-octadecadienoic acid isomers were detected which occur in high concentrations of about 0.1 g/100 g oil. This poster summarizes the analysis and structure elucidation of these newly detected oxylipins.

POSTER 32

OXYLIPIN FORMATION DURING EFFEROCYTOSIS OF DYING HUMAN NEUTROPHILS BY MACROPHAGES

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We have recently shown that pro-inflammatory macrophages differ substantially from wound-healing / anti-inflammatory / pro-resolving macrophages in their profile of released oxylipins. While pro-inflammatory cells primarily release 5-lipoxygenase (LO) products [5(S)-HETE, leukotriene B4] and prostaglandins, anti- inflammatory cells show a profound switch to mono- and dihydroxylated 15-LO-dependent oxylipins such as 15(S)-HETE, 17(S)-HDHA, 5(S),15(S)-diHETE and RvD5 [7(S),17(S)-diHDHA. In contrast, release of the controversially discussed trihydroxylated oxylipins RvD1/2, RvE1/2 was barely detectable to non-existent [1]. During inflammation resolution, a subset of macrophages removes dying neutrophils accumulating at the site of inflammation together with other cell debris. This process, known as efferocytosis, is a key step in the resolution of inflammation and contributes to macrophages developing an anti-inflammatory / pro-resolving phenotype. Interestingly, oxylipin formation during efferocytosis of neutrophils by macrophages has never been studied in detail. We therefore decided to investigate this process in vitro. For this purpose, monocytes were isolated from human peripheral blood and subsequently differentiated into macrophages with M-CSF for 7 days. In addition, Interleukin-4 was added in the last two days of the incubation to induce the M2 phenotype. After differentiation, the cells were co-incubated with dying neutrophils for several hours. Neutrophil apoptosis was induced by serum deprivation during overnight incubation. Co-incubation with apoptotic cells was compared with necrotic neutrophils resulting from several freeze-thaw cycles and with healthy cells from the same donor. Subsequently, oxylipin formation in the cell supernatants was analyzed by LC/MS-MS technique.

1. Ebert R, Cumbana R, Lehmann C, Kutzner L, Toewe A, Ferreirós N, Parnham MJ, Schebb NH, Steinhilber D, Kahnt AS. Long-term stimulation of toll-like receptor-2 and -4 upregulates 5- and 15-LO-2 expression thereby inducing a lipid mediator shift in human monocyte-derived macrophages. Biochim Biophys Acta Mol Cell Biol Lipids. 2020 Sep 1865(9):158702.

POSTER 33

IMAGING LIPOPROTEIN(A) AND ITS LIPID CARGO IN ATHEROSCLEROSIS USING MASS SPECTROMETRY

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Background: Lipoprotein(a) (Lp(a)) is a known risk factor for atherosclerosis, but its exact role in disease progression remains elusive. Although previous studies have studied Lp(a)'s association with atherosclerosis at the protein level, studies have not yet explored the spatial distribution of Lp(a) and its lipid cargo in atherosclerotic lesions using mass spectrometry imaging (MSI).

Objective: To investigate the spatial distribution of Lp(a) and its lipid cargo in atherosclerotic lesions using spatial proteomics approach by MSI.
Methods: Imaging experiments were carried out on a MALDI SYNAPT G2-Si mass spectrometer in positive(CHCA matrix) and negative (1,5-DAN matrix) ionisation modes, with the mass range of m/z 100-1200. Lipids were identified using MS/MS and high mass resolution FT-ICR MS data in combination with the LIPID MAPS database. Spatial analysis was performed using Lipostar® and MetaboAnalyst 5.0®. For spatial proteomics MSI, we are optimising trypsin digestion to analyse the proteolytic peptides of Lp(a) with confirmation through Mascot®.

Results: Using spatial lipidomics, we have identified different lipid species that are localized in areas associated with disease progression, such as the necrotic core of atherosclerotic lesions. The results we aim to generate following spatial proteomics will provide novel insights into the role of Lp(a) in atherosclerosis through co-registration of lipidomic and proteomic datasets and shed light on the significance of this protein and its lipid cargo in the development of the disease.

Conclusion: The findings of this study may have implications for the development of novel diagnostic and therapeutic strategies for atherosclerosis through modulating Lp(a).

POSTER 34

STEROL DERIVATIVES SPECIFICALLY INCREASE 15-LOX PROTEIN ABUNDANCE AND 15-LOX-CATALYZED OXYLIPIN FORMATION IN HUMAN M2-LIKE MACROPHAGES

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The crucial role of the liver X receptor (LXR) in macrophage regulation during inflammation has emerged in recent years. The LXR belongs to a family of nuclear receptors involved in the regulation of metabolic homeostases as well as inflammation. Besides, lipid mediators, cytokines and chemokines play a key role in the regulation of these inflammatory processes. Recently, we reported that the synthetic LXR agonist T09 together with interleukin 4 (IL-4) increases the gene expression ALOX15 in human M2-like macrophages. Its protein product, the15-lipoxygenase (15 LOX) catalyzes oxylipin formation from polyunsaturated fatty acids. The resulting products contribute to the regulation of inflammatory processes.

In this study, we analyzed oxylipins and the abundance of oxylipin-forming enzymes in human M0, M1 and M2-like macrophages following LXR activation by synthetic agonist T09. Furthermore, we investigated the effect of endogenous sterol derivatives on the regulation of ALOX15 expression and 15-LOX-catalyzed oxylipin formation. For this, primary human monocyte cells were isolated from buffy coats and differentiated: (i) M1 or M2-like primary macrophages were incubated with CSF-2 or CSF-1 for 8 days and treated with interferon-gamma or IL-4 for the final 48 h; (ii) No cytokines were added to generate M0-like macrophages. The LXR-activation by T09 had no or only moderate effects on the polarization of macrophages based on the abundance of phenotype-specific proteins (TLR2, TLR4, PPARγ and IL-1RII) and surface markers (CD14, CD86 and CD163). The LXR-agonist T09 increased 15-LOX abundance specifically in M2 macrophages: In a time- and dose-dependent manner, protein abundance of 15-LOX as well as the 15-LOX-derived oxylipin 15-HETE were increased up to 3-fold. The dihydroxy-fatty acids 5,15-DiHETE was increased up to 7-fold, while non-15-LOX-derived oxylipins did not change. The sterol derivatives desmosterol, 24(S),25-epoxy cholesterol and 22(R)-hydroxy cholesterol were identified as endogenous LXR-activating ligands, yielding an increase in the expression of ALOX15. With this, we describe a new link between the two lipid mediator classes sterols and oxylipins in macrophages, suggesting that cholesterol precursors play a role in the regulation of inflammation.

POSTER 35

ALOX15B AIDS IN THE RESOLUTION OF PSORIASIS THROUGH MODULATION OF PLASMA MEMBRANE LIPIDS AND EGFR/JAK1/STAT1 SIGNALLING AXIS

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Lipids are essential in cutaneous biology, which is underscored by their importance in the formation of the permeability barrier as well as involvement in modulating inflammation, cell growth and differentiation.
Psoriasis is a chronic inflammatory skin disease affecting approximately 3% of the population worldwide. Moreover, dysregulation in the abundance of biological lipid mediators has been reported in the skin and blood of psoriatic compared to healthy individuals. Arachidonate 15-lipoxygenase type B (ALOX15B) peroxidises polyunsaturated fatty acids to their corresponding fatty acid hydroperoxides. These lipid hydroperoxides are subsequently reduced into anti-inflammatory lipid alcohols. RNA scope and immunofluorescence staining showed an increase in ALOX15B expression in lesional psoriasis samples. Normal human epidermal keratinocytes were used as a model cell system for the siRNA-mediated knockdown of ALOX15B.
Psoriasis-like phenotype was investigated through treatment with a cytokine cocktail containing interleukin-17A, interferon-gamma and tumour necrosis factor-alpha. These data revealed increased ALOX15B expression along with C-C motif chemokine ligand 2 (CCL2) expression and secretion, which was augmented with ALOX15B knockdown. In addition to CCL2, secretion of CCL5 and CXCL10 were elevated in 3D skin equivalents treated with ML-351, an inhibitor of ALOX15B. Western analysis revealed signal transducer and activator of transcription (STAT1) expression was increased following ALOX15B knockdown. Inhibition of Janus kinase I or knockdown STAT1 in ALOX15B knockdown keratinocytes reversed the effect of enhanced CCL2 gene expression. Furthermore, alterations in plasma membrane lipids were detected via confocal microscopy, indicating reduced cholesterol and increased phosphatidylinositol 4,5-bisphosphate. Additionally, epidermal growth factor receptor (EGFR) expression was reduced in ALOX15B knockdown cells.
Inhibition of EGFR revealed an increase in CCL2, CCCL5 and CXCL10. We therefore hypothesise that upregulation of ALOX15B expression aids in the resolution of psoriasis, through modulating inflammatory signalling via alterations in plasma membrane lipids.

POSTER 36

THE LIPOXIN RECEPTOR FPR2/ALX IS ASSOCIATED WITH LOWER INFLAMMATION AND BETTER PROGNOSIS IN HUMAN ACUTE HEART FAILURE

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Background: Dysregulated inflammation contributes to acute heartfailure (AHF) and cardiogenic shock (CS) pathophysiology. The identification of lipoxins (LXs) and their FPR2/ALX receptor as key players in the resolution of inflammation response has led to new potential treatment targets. Since no data on human AHF is yet available, we evaluated both in AHF and CS patients, as well as FPR2/ALX association with cytokines, cardiac markers, prognostic scores and in-hospital survival.

Methods: Blood and urine samples of AHF (n=23) and CS (n=25) patients were collected at admission, days 3-4 and 5-8. Blood donors (BD) were used as controls (n=22). We quantified serum and urinary lipoxin A4 (LXA4) and 15-epi-LXA4 by ELISA and FPR2/ALX receptor expression in peripheral blood mononuclear cells by RT-qPCR. Systemic cytokines (TNF-alpha, IL-1beta, IL-6, IL-10), B-type natriuretic peptide (BNP), high-sensitivity troponin I (hsTnI) and prognostic scores (APACHE II, SAPS II) as well as in-hospital survival were also analyzed.

Results: At admission there were no differences in serum LXA4 or 15-epi-LXA4 between groups. However, both urinary LXA4 and 15-epi-LXA4 were higher in AHF compared to BD ($p<0.001$) and urinary 15-epi-LXA4 was also higher in AHF compared to CS ($p<0.05$). During hospitalization, a decreasing profile was observed only in AHF at days 3-4 vs admission for S-LXA4 ($p<0.05$), S-15-epi-LXA4 ($p<0.05$), U-LXA4 ($p<0.01$) and U-15-epi-LXA4 ($p<0.01$). FPR2/ALX expression was significantly raised at admission ($p<0.01$, CS vs BD) and decreased along hospitalization only in CS (days 3-4 vs admission, $p<0.05$). Noteworthy, in multivariate analyses, FPR2/ALX expression was inversely associated with TNF-alpha (ß=-0.075, $p<0.001$), IL-10 (ß=-0.001, $p<0.001$), BNP (ß=-0.00003, $p<0.05$), hsTnI (ß=-0.00003, $p<0.01$), APACHE II (ß=-0.093, $p<0.01$) and SAPS II (ß=-0.053, $p<0.01$) and positively associated with in-hospital survival (ß=1.298, $p<0.05$), only in AHF patients.

Conclusions: CS patients appear to have defective resolutive response as suggested by unchanged LXs profile and higher FPR2/ALX expression at admission. FPR2/ALX inverse association with cytokines, cardiac markers and prognostic scores as well as its positive association with in-hospital survival only in AHF patients suggest that it exerts a protective role in these patients. Funded by FCT (PTDC/MEC-CAR/32188/2017) & COMPETE, Portugal 2020 (POCI-01-0145-FEDER-032188)

POSTER 37

PHOSPHOLIPID ESTERS FROM HERRING ROE PROMOTES SPM BIOSYNTHESIS IN HUMAN MONOCYTE-DERIVED MACROPHAGES WITH IMPLICATIONS FOR THE TREATMENT OF PSORIASIS

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Phospholipid Esters from Herring Roe (PEHeRo) are polar amphipathic lipids naturally enriched in marine long-chain polyunsaturated fatty acids (LC-PUFAs). DHA and EPA are the most abundant omega-3 LC-PUFAs in PEHeRo and have known involvement in the resolution of inflammation through specialized pro-resolving mediator (SPM) biosynthesis. Prior studies on Herring Roe Oil (HRO) containing PEHeRo (IRIS ID: 300000046327) have displayed promising immunomodulatory functions in vivo, where HRO has been shown to improve mild-to-moderate psoriasis in a clinical trial in humans (n=64). Psoriasis is a multifactorial inflammatory disease associated with keratinocyte hyperproliferation and elevated inflammatory cytokine levels, where the IL-23/IL-17 axis is central. We have investigated the effect of PEHeRo on pro-resolving biosynthetic pathways in IL-23 producing activated human monocyte-derived macrophages (MDM).

Materials and Methods: MDMs were either co-stimulated with 5% (w/w) emulsions of HRO and 1 ng/ml lipopolysaccharide (LPS) for 24 h, or treated with the emulsion for 16 h, followed by change of medium with addition of 1 microgram/ml LPS and 50 ng/ml IFN-gamma for further 24 h. After stimulation, the supernatants were collected and analyzed for lipid mediators using LC-MS/MS.

Results: Our results show a marked upregulation of SPM biosynthesis in the MDMs after stimulation with HRO including E-series resolvins (RvEs), D-series resolvins (RvDs), protectins (PDs), and protein conjugates in tissue regeneration (PCTRs). Several of the upregulated SPMs have been reported to be relevant for resolving psoriatic inflammation, including effects on the IL-23/IL-17 axis.
Interestingly, a purified fraction of PEHeRo from HRO elicit the highest cell response for multiple SPMs in the supernatants. This observation cannot be explained by normalization of the SPM levels to precursor levels in the oils and suggests that the overall composition of the oils, and not only levels of individual fatty acids, influences the SPM activity observed in the MDMs.

Conclusion: Our findings support an anti-inflammatory action of HRO, and PEHeRo specifically, through activating the production of a set of SPMs which promote a shift towards a protective and possibly reparative phenotype of MDMs. This effect could be a promising treatment modality in inflammatory conditions, including psoriasis.

POSTER 38

THE LIPID MEDIATOR LANDSCAPE ASSOCIATED WITH INFECTION AND ORGAN FAILURE IN PATIENTS WITH ADVANCED LIVER DISEASE

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Background and Aim: Patients with advanced liver cirrhosis such as those with acutely decompensated (AD) cirrhosis present immunosuppression, recurrent infections and a hyper inflammatory response causally linked to the development of organ failures, a condition known as acute-on-chronic liver failure (ACLF) characterized by high short-term mortality. In this study, we analyzed the lipid mediator landscape associated with infection and organ failure in patients with AD cirrhosis.

Methods: Lipid mediators were assessed by LC-MS/MS in plasma samples from 1346 patients with AD cirrhosis and 30 healthy subjects. A multifactorial regression network was built to identify significant associations between lipid mediators, infection and ACLF.

Results: The profile of lipid mediators was similar in AD patients with infections that in those without. However, network analysis identified that arachidic acid, a saturated fatty that exerts a mitochondrial uncoupling effect, positively associated with active infections whereas 15-HETE, a pathway marker of resolution of inflammation, negatively associated with this clinical feature. On the other hand, the comparison between AD patients that developed ACLF with those that did not develop ACLF identified three lipid mediators with statistically significant value: tridecanoic acid (a saturated fatty acid produced by bacteria), hydroxydodecanoic acid (hydroxylated medium-chain fatty acid esterified to the lipid A backbone of lipopolysaccharide bacteria), and sphingosine 1 phosphate (S1P, a lipid mediator that controls immune cell trafficking). In contrast to tridecanoic and hydroxydodecanoic acids, S1P levels were strongly and negatively associated with ACLF. Indeed, S1P negatively correlated with coagulation, circulatory and liver failures. Moreover, S1P which was primarily associated with the ramping thrombocytopenia existing in AD patients whereas the rest of the lipid mediators described above were linked to the existing neutrophilia.

Conclusion: Together, these findings identify five lipid mediators differentially regulated in the peripheral circulation of patients with AD cirrhosis which are associated with the presence of infections and the development of organ failures in this condition.

POSTER 39

COMPREHENSIVE, SENSITIVE ANALYSIS OF BIOACTIVE LIPIDS USING METAL-FREE LC-MS/MS

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Signaling lipids like oxylipins, lysophosphatidic acids (LPA), and ceramide-1-phosphates (CerP) are involved in various cellular processes, including cell migration, growth, and death, and are important players in inflammatory processes.[1-2] While approaches have been made to develop an assay for a few of these lipid classes [2], there currently is no comprehensive workflow that can measure all important signaling lipid classes in one run due to challenges like a high dynamic range and, most of all, the different structures and chemical properties of these analytes.[2]

Using standards from 17 different lipid classes, we established a bioinert, targeted LC-MS/MS workflow to analyze close to 400 bioactive lipid species in a single 20-minute run. We tested different column hardware and gradient compositions to reduce the carryover of free phosphate group-containing analytes like CerPs and LPAs. Carryover effects were significantly reduced by choosing the right column hardware and salt and acid concentrations. Sample preparation was optimized and consisted of a one-step MMC (MeOH/MTBE/CHCl3) extraction, enabling robust and high lipid recoveries. The established workflow was validated in platelet and plasma matrix and yielded excellent performance parameters, including LLOQs in the low nM range and repeatability <15% RSD. Applying our workflow to human plasma, we identified 307 lipid species, spanning a dynamic range of 6 orders of magnitude. We furthermore investigated the signaling lipidome during platelet activation and identified 267 lipids, of which 85% were regulated upon stimulation. The high coverage of our established method furthermore enabled the detailed investigation of distinct lipid signaling pathways for glycerophospholipid- derived and sphingoid-based signaling lipids on a molecular lipid species level, providing the first comprehensive report of signaling lipid regulation during platelet activation.

References:

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POSTER 40

PRESERVATION OF AGE- AND SEX-SPECIFIC CHARACTERISTICS IN PRIMARY PHAGOCYTES DURING LONG-TERM IN VITRO CULTURE DEPENDS ON THE ORIGIN OF SERUMSUPPLEMENT.

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Monocyte lineage-derived phagocytes and tissue-resident macrophages possess potent immunomodulatory abilities and high plasticity to adapt to their microenvironment and intrinsic factors like biological age and sex. In vitro culture of primary phagocytes is inseparable from supplementation with serum, which provides necessary nutrients, hormones, and growth factors. Although individual disparities in innate immunity are an emerging research topic, validated in vitro models that allow long-term assessment of cellular characteristics of cultivated phagocytes considering both biological age and sex are scarcely available. Here, we show sex-, age- and origin-specificity of serum, particularly in their respective content of oxylipins, sexual hormones and trace elements. Cultivation with serum from human, murine or bovine origin causes differential expression of surface markers and oxylipin biosynthetic enzymes in primary phagocytes of various origin. We elucidate sex-specific phenotypes for mononuclear phagocytes isolated from human donors which are preserved through supplementation of sex-matched human serum (HS) and abolished by the usage of fetal calf serum (FCS). Additionally, we provide sex-specific oxylipin profiles for human phagocytes after bacterial infection that are conserved under the influence of sex-matched HS but absent in the presence of FCS. Furthermore, sex-specific differences in phagocytosis of bacterial debris between different human phagocyte subsets only become apparent when employing HS. Finally, we reveal sex-specific aspects of inflammaging in the oxylipin response of murine resident macrophages from the peritoneal cavity by supplementation of sex- and age-matched murine serum prior to bacterial infection.

Taken together, our data emphasize a multi-facetted sex- and age-bias in the plasticity and functionality of primary phagocytes, highlighting the importance of precise selection of experimental conditions to adequately address sexual dimorphism and consequences of aging.

POSTER 41

MEMBRANE-BOUND OXYLIPINS ARE MARKERS OF OXIDATIVE STRESS

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Many diseases including the leading causes of death in western societies such as cardiovascular diseases, chronic inflammation and cancer are accompanied by oxidative stress. Oxidative stress means the imbalance between the production of reactive oxygen and nitrogen species (RONS) and their degradation by cellular protective mechanisms. Misregulation leads to a massive increase in reactive oxygen species which cause damage to biomolecules like DNA, proteins and membrane lipids. The oxidation of lipids by lipid peroxidation leads to the formation of a multitude of oxylipins and secondary products. Due to the stereo random reaction mode of autoxidative lipid peroxidation, a vast spectrum of oxylipins is formed.

Besides isoprostanes, which are established markers, other oxylipins esterified to phospholipids in cellular membranes can be useful and sensitive read-outs for oxidative stress. Some years ago, the ratio of trans- versus cis-epoxy fatty acids was characterized as new marker for oxidative stress in vitro and in vivo (Prostag Oth Lipid M, 2019, 144, 106334).

In the present work, we investigate the oxylipin pattern in different models of oxidative stress using cell lines such as the liver cancer cell line HepG2 challenged with i.) the direct radical generating tert-butylhydroperoxide, ii.) the redox cycler paraquat, iii.) rotenone (an inhibitor of complex I in the electron transport chain) as well as iv.) the GPX4 inhibitor RSL-3.
The pattern of non-esterified as well as esterified oxylipins is quantitatively measured by state-of-the-art targeted metabolomics. Following alkaline hydrolysis of esterified oxylipins (Prostag Oth Lipid M, 2020, 146, 106384), oxylipins are extracted by means of solid phase extraction and analyzed by liquid chromatography- tandem mass spectrometry (Anal Chim Acta, 2018, 1037, 63-74).

On the poster, we demonstrate that esterified oxylipins, including isoprostanes and a distinct pattern of oxylipins allow to sensitively detect oxidative stress. These could serve as new markers characterizing cellular pathways leading to elevated RONS production.

POSTER 42

MAPPING HEPATIC LIPID DYNAMICS: A MASS SPECTROMETRY IMAGING APPROACH TO ASSESS METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE

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Metabolic dysfunction-associated steatotic liver disease (MASLD) refers to a range of conditions involving the accumulation of excess fat in the liver. Metabolic dysfunction-associated steatohepatitis (MASH) is the progressive, inflammatory form that can lead to fibrosis, cirrhosis, and liver cancer. We hypothesized that mass spectrometry imaging (MSI) can be used to generate spatial information about lipid accumulation in MASH. Our aim was to develop a robust MSI method to spatially characterize hepatic lipidome profiles, identifying novel biomarkers and potential therapeutic targets for MASH. Livers from an atherogenic model of 12 male ApoE-/- mice, aged 19 weeks and fed a high-fat diet for 12 weeks to induce MASH and fibrosis, were cryosectioned at 10 µm thickness for MSI. Matrices were applied using an HTX TM-SprayerTM, and tissue samples were analyzed using matrix-assisted laser desorption/ionization (MALDI)-interfaced with a SYNAPT G2-Si QToF instrument (Waters). Method optimization was performed in both positive and negative ion modes, including the selection of suitable matrices, solvents, matrix spray parameters, and instrument conditions. Matrices, 2,3-dicyanohydroquinone (DCH, 10 mg/mL; density=0.0055 mg/mm^2) and 1,5-diaminonaphthalene (DAN, 5 mg/mL; density=0.0019 mg/mm^2) in 70% acetonitrile v/v, analysed at 75 µm pixel size, yielded high spatial resolution images and were chosen for lipid extraction from mouse liver tissues in positive and negative ionization modes, respectively. Data analysis employed high definition imaging (HDI), MassLynx, and Lipostar-MSI software.

The optimized method identified various lipid species, including phospholipids such as PG (phosphatidylglycerol), PA (phosphatidic acid), and PC (phosphatidylcholine). The intra- and inter-day reproducibility tests of the MSI method demonstrated less than 20% variation in relative standard deviation, ensuring reliable lipidomic analyses. This MSI approach will enable the study of the hepatic lipidome in inflamed and fibrotic regions, furthering our understanding of MASH pathogenesis and potential therapeutic interventions.

POSTER 43

DYSREGULATION OF RESOLVIN E1-CHEMERIN1 AXIS IN CRITICAL COVID-19 PATIENTS

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Background: In contrast to the plethora of studies evaluating cytokines in COVID-19, few studies in these patients investigated proresolving pathways, including specialized proresolving lipid mediators such as resolvins as well as their receptors. Thus, among a panel comprising cytokines, resolvins and their receptors, we evaluated which mediators best differentiate: (1) controls and hospitalized COVID-19 patients; (2) severe and critically ill COVID-19 patients; (3) COVID-19 patients requiring or not mechanical ventilation; and (4) survivors and non-survivors. Methods: Serum cytokines (IL-1beta, IL-6, IL-10, TNF-alpha, IFN-gamma, GM-CSF) as well as serum resolvins (RvD1; RvE1) and their respective receptors (FPR2/ALX; chemerin1) mRNA content in peripheral blood mononuclear cells were measured by multiplex immunoassays, ELISA and RT-qPCR, respectively, in “severe” (n=27), “critical” (n=17) and critical on veno-venous extracorporeal membrane oxygenation (“VV-ECMO”; n=17) COVID-19 patients at admission, days 3-4 and days 5-8, and in controls (n=23) at a single time point. Principal component analysis, partial least squares discriminant analysis, univariate and correlation analyses were performed using SIMCA 14 and GraphPad Prism 10. Results: When considering all above mentioned variables, we obtained significant models that differentiated: (1) controls from all patients and each patient group ($p<0.001$); (2) “severe” from all critical patients ($p<0.010$); (3) patients without or with mechanical ventilation ($p<0.001$) and (4) survivors from non-survivors ($p<0.001$). RvE1 consistently showed a VIP score>2.5 and a p(corr)>0.8, being the most relevant variable discriminating the groups. In fact, in univariate analyses, RvE1 was increased in all patient groups ($p<0.001$ vs controls), being even higher in “VV-ECMO” patients, while chemerin1 was lower in the patient groups ($p<0.050$ or $p<0.001$ vs controls), especially in “VV-ECMO”. Interestingly, mechanically ventilated patients and non-survivors presented significantly higher RvE1 and lower chemerin1. Finally, RvE1 positively correlated with IL-1beta, IL-6, IFN-gamma, GM-CSF, neutrophil-to-lymphocyte ratio (NLR) and PaCO2, whereas chemerin1 inversely correlated with NLR, PaCO2 and hospitalization time and positively correlated with PaO2/FiO2 ratio.

Conclusions: RvE1 was the inflammatory mediator that best distinguished COVID-19 disease severity. RvE1 upregulation, accompanied by the downregulation of its receptor, mostly in critical patients, suggests a failure of this proresolving axis in these patients.

Funded by FCT-RESEARCH 4 COVID-19 grant, proj-519-ref-613690173

POSTER 44

BISPHENOL-A-INDUCED INFLAMMATORY RESPONSE IS ASSOCIATED TO SPHINGOLIPID METABOLISM ACTIVATION IN AIRWAY EPITHELIAL CELLS.

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Epidemiologic studies indicate that exposure to endocrine-disrupting chemicals, including bisphenol A (BPA) and phthalates is associated with asthma morbidity which may differ by sex. Recently we have demonstrated that polymorphisms in orosomucoid-like 3 (ORMDL3) proteins, which play a crucial role in sphingolipid homeostasis and synthesis, are associated with an increased risk of asthma in a sex-related manner. In particular, the main sphingolipid metabolite, sphingosine-1-phosphate (S1P), has been identified as a critical player in asthma in pre-clinical and clinical settings. S1P is an intracellular product formed in multiple cell types in response to numerous stimuli including antigens and cytokines. It is involved in various cellular processes, including cell growth, differentiation, proliferation, signal transduction, and immune response. The aim of this study is to investigate the effect of BPA on sphingolipid metabolism in human type II alveolar epithelial A549 cells. Cells were exposed for 24h to BPA (0,1-1μM) and cell viability was evaluated through MTT assay; reactive oxidant species (ROS) by means of the probe 2', 7'-dichlorofluorescein-diacetate; expression of cyclooxygenase-2 (COX-2), vimentin, a-SMA, ORMDL3, ceramidase, and S1P receptor, by western blot or immunofluorescence (IF) analyses. BPA induced in epithelial cells an inflammatory phenotype with an increase in ROS production and COX-2 expression in a concentration-dependent manner. BPA also stimulated the Epithelial-Mesenchymal Transition (EMT) of A549 cells, characterized by a concentration-dependent increase of mesenchymal markers such as vimentin and a-SMA. These effects were coupled to the upregulation of all pathways involved in the activation of sphingolipid metabolism. In addition, BPA increased both ORMDL-3 and the ceramidase that converts ceramide to sphingosine. In perfect tune, the IF of BPA-treated A549 showed an upregulation of S1P receptor S1P2.

In conclusion, our data demonstrate that BPA induces lung epithelial activation and inflammatory response associated with sphingolipid pathway activation.

POSTER 45

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

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N-acylethanolamines (NAEs) constitute a family of lipid mediators that comprises, among other members, the endocannabinoid N-arachidonoylethanolamine (AEA, also known as anandamide) and the anti- inflammatory lipid N-palmitoylethanolamine (PEA). Many studies have demonstrated the key role of these lipid mediators in several physiological and pathological settings. Several biochemical pathways are responsible for the production of NAEs with the key precursors for NAE biosynthesis being the 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 (notably in the context of obesity). Strikingly, only little is known on the biological effects of NAPEs and on how their levels are changed in physiological and pathological contexts. This is in part due to the limited number of described methods allowing quantifying NAPEs.

As NAEs and NAPEs are biochemically linked, we thought it would be interesting to develop an LC-MS/MS method allowing to quantify both NAPEs and NAEs in biological settings.

First, using NAPE and NAE standards, we developed a chromatographic UPLC method based on a gradient between water and methanol and investigated the effect of solvent additives (e.g. ammonium acetate). We also optimized the parameters of the Waters Xevo TQ-s mass spectrometer. One of the objectives of this step was to be able to identify which NAE moiety was present on the analyzed NAPE as this informs on the resulting NAE that will be biologically produced from that specific NAPE.

Next, we set up the extraction procedure and the silica-based solid phase extraction (SPE) pre-purification step in order to obtain, within the same SPE fraction, both the NAEs and the NAPEs. One of the aims here was to remove the more lipophilic lipids and then elute the analytes of interest while leaving more polar compounds on the column.

After it's validation, the developed method will allow us to follow the variations in NAE and NAPE levels in biological matrices.

POSTER 46

AN INVESTIGATION OF KEY INFLAMMATORY CELLS AND RECEPTORS IN ZEBRAFISH LARVAE FOLLOWING INJURY AND REGENERATION

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Inflammation is an essential physiological response for defense against infectious agents, reaction to trauma and for restoration of tissue homeostasis. It currently stands that the majority of research is centered around the onset and propagation of inflammation, whereas the resolution of inflammation needs to be investigated more thoroughly. Neutrophils are terminally differentiated granulocytes, and monocytes circulate in the blood and migrate into tissues to differentiate into macrophages. Macrophages tend to accumulate after the initial waves of neutrophils have migrated to the site of trauma. These leukocytes are known to respond to both DAMPs and PAMPs. The processes of initiation, propagation and resolution of inflammation are modulated by key receptors such as the leukotriene B4 receptor (BLT1) and the cannabinoid receptors (CB1 And CB2).

Zebrafish are becoming ever more prevalent within the biomedical research world for a number of reasons and have been used within this project to explore the roles of the aforementioned lipid receptors on neutrophils and macrophages, along with tissue regeneration. Larval zebrafish tailfin transection and spinal cord injury models have been optimised and show an initial rise in neutrophil numbers within the first 8 hours post-injury, and a peak within the macrophage numbers at around the 24-hour mark, with both cell types returning to baseline.

In addition, compared to the controls post-tail fin injury, it has been shown for the first time that bathing the larvae in the presence of leukotriene B4 caused a striking augmented increase in both neutrophil and macrophage numbers that occurs in a concentration- and time-dependent manner. Similar work is being conducted on a CB2 inverse agonist called Sch336. Human-based flow cytometry work to fully analyse the role of the cannabinoid and leukotriene receptors in inflammation and tissue regeneration is underway. This work sets the scene for investigation of mediators together with the study of leukocytes in the above models.

POSTER 47

ACCURATE SPHINGOLIPID QUANTIFICATION REDUCING FRAGMENTATION BIAS BY NONLINEAR MODELS

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Quantitative sphingolipid analysis is crucial for comprehending the roles of these bioactive molecules in various physiological and pathological contexts. Molecular sphingolipid species are typically quantified using sphingoid base-derived fragments relative to a class-specific internal standard. However, the commonly employed “one standard per class” strategy fails to account for fragmentation differences presented by the structural diversity of sphingolipids. To address this limitation, we have developed a novel approach for quantitative sphingolipid analysis. Starting from a previously developed LC-ESI-MS/MS-based approach for the comprehensive analysis of sphingolipids in biological samples, we focused on optimizing the quantification in terms of post-acquisition data correction. Using only one internal standard for the entire lipid class, we developed a fragmentation model for ceramides as an example, which may be extended to many other lipid classes. This approach uses the information on double bonds and hydroxy groups for a rough calculation of the correction factor and the chain length for a more precise one. The determined response factors are based on experimental data and are independent of the employed instrumentation, collision energies or matrix, and may be extended to another internal standard. To automatize data processing after acquisition, such as calculating species-specific correction factors, a workflow was developed using the software tool “Konstanz Information Miner” (KNIME). Overall, the workflow has proven suitable for processing data from lipidomic analyses of complex biological samples, such as fat cells, giving the same quantitation results, as manually obtained from the same data set, in 1/100 of the time.

POSTER 48

MEASUREMENT OF OXYLIPINS IN BLOOD FROM PATIENTS WITH HYPERTENSION

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Arterial Hypertension (HTN), a multifactorial disorder resulting from the interplay between genetic predisposition and environmental risk factors, is a common comorbidity in patients with increased metabolic risk or manifest metabolic syndrome, affecting up to 40% of individuals with non-alcoholic fatty liver disease (NAFLD).

In this pilot study, we assessed 57 patients presenting to a metabolic outpatient clinic and determined whether patients with HTN had different oxylipin profiles as compared to differed from those without HTN. Patients were characterized by analyzing routine lipid blood parameters, medical history, as well as clinical characteristics. Patients were divided into with hypertension group (n=31) and without hypertension group(n=26). Profiles of PUFAs in blood and their oxylipins in plasma were measured by gas chromatography and liquid chromatography/tandem mass spectrometry. Transient elastography (TE) was used to assess hepatic fat content measured as controlled attenuation parameter (CAP) (in dB/m) and the degree of liver fibrosis measured as stiffness (in kPa).
We found significantly higher age, body mass index (BMI), triglyceride levels, as well as hepatic fat content measured as CAP in patients with hypertension as compared to those without hypertension. Furthermore, 19.20-EDP, 20-HEPE, and 22-HDHA were significantly higher in patients with hypertension, but 11,12-DHET and 9-HODE were significantly lower.

Conclusions: In adult patients presenting to a metabolic clinic, hypertension was associated with increased age, BMI, triglycerides, and liver fat content. These patients also had significantly higher plasma levels of several EPA-and DHA-oxylipins.

POSTER 49

Glucocorticoids cause reciprocal modulation of 15-lipoxygenase isoforms affecting oxylipin biosynthesis involved in inflammation and its resolution

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Glucocorticoids (GC) are potent anti-inflammatory agents, broadly used to treat acute and chronic inflammatory diseases. Thereby, GC not only limits inflammation but also promote its resolution, although the underlying mechanisms are still obscure. Deciphering the cellular and molecular mechanisms of GC actions to develop a more specific and safer anti-inflammatory treatment is of utmost importance. Here, we reveal reciprocal regulation of 15-lipoxygenase (LOX)-1 and -2 isoform expression in human monocyte/macrophage lineages by GC with respective consequences for the biosynthesis of inflammation-related oxylipins.[1] The 15-LOX-1/-2 isoforms are key enzymes in the oxygenation of polyunsaturated fatty acids (PUFAs) towards specialized pro-resolving mediators (SPMs) and corresponding mono-hydroxylated precursors (mono-15-OH) with anti-inflammatory properties. Dexamethasone (Dex) robustly upregulated mRNA and protein levels of ALOX15B/15-LOX-2 in different monocyte-derived macrophage (MDM) phenotypes, causing elevated SPM and mono-15-OH production and lowered levels of prostaglandins and leukotrienes in inflammatory cell types. On the other hand, 15-LOX-1/ALOX15 upregulation by IL-4 in pro-resolving M2-like MDM was abolished when cells were treated with Dex, thereby lowering the amounts of SPMs and mono-15-OH. The actions of Dex were mimicked by prednisolone and hydrocortisone, and they were counteracted by the GC receptor (GR) antagonist RU486. Chromatin immunoprecipitation (ChIP) assays revealed robust GR recruitment to a putative enhancer region within intron 3 of the ALOX15B gene. Finally, ALOX15B/15-LOX-2 upregulation was evident in human monocytes from GC-treated patients with COVID-19 or patients with inflammatory bowel disease. Our findings underline the benefit of GC in acute and severe inflammatory reactions and support their use in early rather than in the later stages of inflammation progression.

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POSTER 50

SELENIUM MODULATES THE REDOX HOMEOSTASIS OF HUMAN PRO-RESOLVING MACROPHAGES AND DRIVES SEX-SPECIFIC 15-LIPOXYGENASE-DEPENDENT LIPID MEDIATOR FORMATION

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The trace element selenium plays a pivotal role in the modulation of the immune response by regulating the redox state. As a component of selenoproteins, selenium helps maintaining the cellular redox balance in macrophages, influencing the efficacy of various macrophage functions, including lipid mediator (LM) formation and phagocytosis. Here, we examined sex-specific disparities in the redox state and the production of LM and phagocytosis upon physiological supplementation of selenium. By using sex-matched human serum supplementation throughout the entire cell culturing process, we received marked sex-specific LM profiles after bacterial stimulation. Our study reveals a sex-specific upregulation of 15-lipoxygenase (LOX)-derived LM in response to selenium supplementation within human pro-resolving macrophages (M2a). In M2a from females, we observed a selenium-dependent increase in the formation of 15-LOX-derived LM, which did not occur in cells from males. Additionally, we provided a mixture of EPA and DHA as exogenous substrates to M2a in order to investigate whether the observed responses are due to divergent substrate supply, and found the same female-specific increase in LM formation. Protein expression of 15-LOX-1 in M2a, however, remained unaltered by selenium in cells of either sex. Analysis of the phagocytic capability of macrophages showed an increase in cells from female donors and a significant decline in male counterparts post-selenium supplementation. Additionally, we observed a female-exclusive reduction in lipid peroxidation despite comparable expression levels of glutathione peroxidases in cells of both sexes. These results unveil sex-specific responses in the formation of 15-LOX-derived LM in dependence of the selenium status of the donors, underscoring the necessity to further studying this complex interplay in a sex-dependent manner. Together, our findings provide novel insights into the regulation of 15-LOX in human macrophages, and emphasize the importance of adequate selenium supply, particularly among females.

POSTER 51

SEX-RELATED ROLE OF LEUKOTRIENES TO SPHINGOSINE-1-PHOSPHATE-INDUCED ASTHMA-LIKE FEATURES

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Asthma is a chronic disease of the airways. Furthermore, there is a clear sex disparity in asthma, as well as therapeutic efficacy in asthma varies between males and females. Sex hormones play a crucial role in shaping the differences in asthma prevalence between males and females during the transition from childhood to adulthood. Sphingosine-1-phosphate (S1P) has been identified as a significant contributor to asthma in preclinical and clinical settings. The sphingolipid metabolism is significantly affected in asthma and the S1P levels increase and correlate with the severity of the disease (1). Leukotrienes (LTs) are lipid mediators involved in asthma pathogenesis, and we have recently demonstrated sex disparities in LT biosynthesis and anti-LT pharmacology in inflammation (2,3,4). Thus, this study aims to investigate the role of S1P in airway hyperresponsiveness (AHR) and the interaction with the LT pathway in the lung. For this purpose, male and female BALB/c mice were sensitized to ovalbumin (OVA) or exposed to systemic administration of S1P. Bronchi and pulmonary tissues were harvested for functional and molecular studies. Part of the mice were pretreated with L-cycloserine, an inhibitor of sphingolipid metabolism, or LT biosynthesis inhibitor, MK886. BALB/c mice exposed to OVA or S1P display sex-related asthma feature development in favour of females coupled to a higher Th-2 immune response and LT production. This sex dimorphism was associated with significant differences in AHR, plasma IgE, and IL-5 pulmonary levels. L-cycloserine treatment inhibited all asthma features similarly to MK886 in OVA-sensitized mice in a sex-dependent manner. Further, the pretreatment with MK886 reduces S1P-induced asthma-like features only in females by reducing IgE plasma level and Th-2 cell recruitment. In conclusion, these results suggest the existence of a sex-dependent functional interaction between S1P and LT signalling and the molecular basis of a sex-tailored therapy in asthma.

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POSTER 52

INVESTIGATING THE PROSTAGLANDIN E2/IL-22 PATHWAY IN ECZEMA

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Background: Eczema, the commonest inflammatory skin condition, is a clinically heterogeneous disease whose pathogenesis relies on the interplay between immune, genetic and environmental factors. It is caused by predominantly type 2 and 17 immune responses (involving Th2, Th17 and Th22 cells) as well as a defective terminal differentiation of keratinocytes. Having previously demonstrated that the bioactive lipid mediator, prostaglandin E2 (PGE2), promoted lymphocyte production of IL-22 from Th22 cells in a mouse model of eczema, we now wish to determine whether this occurs in eczema patients.

Methods: CD3+ T cells were obtained via cell separation from whole blood following donation by healthy volunteers and were stimulated using CD3/CD28 antibodies, cultured with/without PGE2 in order to study the effect of PGE2 on the numbers of IL-22 and IL-17 producing T cells. Using publicly available datasets, we studied the correlation between PGE2/IL22 gene expression for a range of diseases including eczema. Furthermore, following ethical approval we undertook RNA seq of skin biopsies (lesional and non-lesional) from our acute and chronic eczema patients to determine the pathways which define these subgroups.

Results: Fluorescence-activated cell sorting demonstrated that PGE2 significantly promoted increased differentiation into IL-22 and IL-17 producing CD4+ T cells compared to controls. Gene data mining studies from publicly available datasets showed that there was a strongly significant positive correlation between PGE2 and IL-22 pathway gene expression in inflammatory conditions including eczema, a finding we are investigating further within our RNA seq dataset. In our data, expression of pathways such as “cytokine activity” are mainly determined by inflammatory status (lesional vs. non-lesional) while the expression of extracellular matrix genes separates acute inflammation from chronic inflammation.

Conclusion: In summary, our results suggest that the PGE2 /IL-22 pathway plays an important role in eczema pathogenesis although further analysis of our RNA seq data and validation of our findings is required in order to more clearly define the subgroups within this heterogenous disease.

POSTER 53

EFFECTS OF OMEGA-3 ON HUMAN CORONARY VASCULAR TONE INDUCED BY NEUROTRANSMITTERS

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Background: Coronary artery diseases are characterized by chronic inflammatory status and endothelial dysfunction. This involves an increased production of neurotransmitters such as serotonin (5 HT) and acetylcholine. On the other hand, inflammation increases levels of pro-inflammatory lipid mediators such as PGE2 and TxA2. These changes are associated with effects on the vascular function by increasing vasoconstriction. Specialized pro-resolving lipid mediators (SPM), derived from omega-3 polyunsaturated fatty acids: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) play an active role in the resolution of inflammation. Recent results from our group show that DHA and metabolites (Resolvin D1, D5 and Maresin 1) reduce contractions of human coronary arteries (HCA) induced by PGE2 [1]. On the other hand, RvD5 and Mar1 production by human vagus nerve has been measured [2], their impact on the cardiac neuronal system remains unexplored.

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

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

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

Conclusion: Our preliminary results indicate that omega-3 may have an effect on neuronal control of HCA vascular tone, suggesting potential innovative therapeutic strategies.

POSTER 54

5-LOX ACTIVATING PROTEIN: A NOVEL TARGET TO BOOST RESOLUTION OF NEURO-INFLAMMATION IN MS

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Multiple sclerosis (MS) is a devastating neurological disease and one of the most prevalent autoimmune disorders in the Western world. Phagocytes like macrophages and microglia play a dual role in lesion progression by promoting both inflammation and remyelination. The resolution of inflammation is regulated by bio-active lipid mediators (LMs), that are produced by enzymes such as lipoxygenases (LOX). We observed that, in MS lesions 5-LOX expression was present in all CNS cell types. Interestingly, 5-LOX activating protein (FLAP) was increased locally in active lesions and was mostly confined to microglial cells suggesting that activation of the 5-LOX pathway through FLAP under inflammatory conditions is responsible for a detrimental LM shift possibly causing impairment of resolution and enhancing lesion progression. We now found that pharmacological inhibition of FLAP halts myelin induced foam cell formation and reduces proinflammatory cytokine production in LPS activated microglia in vitro. In line with these results, we show that FLAP inhibition promotes remyelination in a microglia-dependent manner and reduces the disease score in well-established ex vivo cerebellar brain slice and in vivo experimental autoimmune encephalomyelitis models.

Altogether, obtained results identified FLAP as a novel target to restore the LM balance, thereby preventing neuro-inflammation and promoting repair in MS and other neurological diseases characterized by the presence of chronic inflammation and demyelination.

POSTER 55

INSIGHTS INTO PGE2-MEDIATED EP4 RECEPTOR ACTIVATION EXPLORED BY MOLECULAR SIMULATIONS

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Lipid mediators, such as prostaglandin E2 (PGE2), play fundamental roles in cellular communication and in the development of diseases like cancer. The lipid mediators interact with specialized proteins called G
protein-coupled receptors (GPCRs), with Prostaglandin E2 Receptor Type 4 (EP4) being particularly significant in cancer and inflammation, driving tumor growth, metastasis, and immune evasion. By employing advanced Free Energy Perturbation (FEP) and atomistic Molecular Dynamics (MD) simulations of the receptor-G proteins complex in its native membrane, we study here howPGE2 activates EP4, offering novel molecular insight into the treatment of diseases. In this regard, we unravel the complex interactions between PGE2 and EP4, revealing mechanisms behind receptor and G protein activation process. Our combined findings provide insights into the functional dynamics of for GPCRs for the future development of innovative therapeutic strategies for cancer by targeting key cellular signalling pathways.