Book of Abstracts

8th European Workshop on Lipid Mediators

Stockholm, June 29 – July 1, 2022

https://workshop-lipid.eu

Platinum Sponsors

MARCUS WALLENBERG FOUNDATION FOR INTERNATIONAL SCIENTIFIC COLLABORATION

Larodan
Research Grade Lipids

AstraZeneca

Gold Sponsors

resolve inflammation
ambiotis

United Therapeutics
CORPORATION

Major Sponsors

Silver Sponsors

Bronze Sponsors

Echelon Biosciences

tebubio
Facilitators of Life Sciences Research

Lab Supplies Nordic AB

ILLUMINATE THE SCIENCE
GOED CLINICAL STUDY DATABASE

ELSEVIER

PROSTAGLANDINS & other LIPID MEDIATORS

Organizing Committee

Mireille ALHOUAYEK
Bioanalysis and Pharmacology of Bioactive Lipids
Louvain Drug Research Institute, Université catholique de Louvain
Bruxelles, Belgium
mireille.alhouayek@uclouvain.be

Gerard BANNENBERG
Global Organization for EPA and DHA Omega-3s (GOED)
Madrid, Spain
gbannenberg@gmail.com

Sven-Erik DAHLÉN
Karolinska Institutet
Institute of Environmental Medicine, Experimental Asthma & Allergy Research
Stockholm, Sweden
sven-erik.dahlen@ki.se

Jesper Z HAEGGSTRÖM
Karolinska Institutet
Dept. of Medical Biochemistry & Biophysics
Stockholm, Sweden
jesper.haeggstrom@ki.se

Per-Johan JAKOBSSON
Karolinska Institutet
Department of Medicine, Rheumatology
Stockholm, Sweden
Per-Johan.Jakobsson@ki.se

Cristina LOPEZ-VICARIO
Hospital Clinic, Inflammation and Liver Disease
University of Barcelona
clopezv@clinic.cat

Giulio G. MUCCIOLI
Bioanalysis and Pharmacology of Bioactive Lipids
Louvain Drug Research Institute, Université catholique de Louvain
Bruxelles, Belgium
giulio.muccioli@uclouvain.be

Xavier NOREL
Eicosanoids and Vascular Pharmacology
INSERM U1148, Bichat Hospital
Paris, France
xnorel@hotmail.com

Olof RÅDMARK
Karolinska Institutet
Dept. of Medical Biochemistry & Biophysics
Stockholm, Sweden
olof.radmark@ki.se

Chengcan YAO
University of Edinburgh
Edinburg, UK
Chengcan.Yao@ed.ac.uk

Local Committee

Magnus BÄCK
Karolinska Institutet
Dept. of Medicine, Cardiovascular Medicine
Stockholm, Sweden
magnus.back@ki.se

Louise BERG
Karolinska Institutet
Department of Medicine, Rheumatology
Stockholm, Sweden
louise.berg@ki.se

Kristina EDFELDT
Karolinska Institutet
Department of Medicine, Rheumatology
Stockholm, Sweden

Marina KOROTKOVA
Karolinska Institutet
Department of Medicine, Rheumatology
Stockholm, Sweden
marina.korotkova@ki.se

Craig WHEELOCK
Karolinska Institutet
Institute of Environmental Medicine, Integrative Metabolomics
Stockholm, Sweden
cewheelock@gmail.com

ORAL PRESENTATIONS

1st sponsored session

COMPARATIVE ROLE OF PROSTACYCLIN IN THE RELAXATION INDUCED BY ATP AND ACH IN PULMONARY ARTERIES FROM PATIENTS WITH OR WITHOUT PULMONARY HYPERTENSION.

A. HICHEM BADJI[1], Heba Abdelazeem[1,2], Dan Longrois[1,3], Adam Silverstein[4] and Xavier Norel[1]

1) Université Paris Cité and Université Sorbonne Paris Nord, INSERM, LVTS, F-75018 Paris, France
2) Department of Pharmacology and Toxicology, Faculty of Pharmacy, Alexandria University, Egypt
3) Hôpital Bichat-Claude Bernard, AP-HP, Université Paris Cité, Paris, France
4) United Therapeutics Corporation, Research Triangle Park, NC 27709, USA

Background: Pulmonary hypertension (PH) is a disabling chronic disorder of the pulmonary vasculature. It is characterized by endothelial dysfunction that leads to a reduction of prostaglandin I_2 (PGI_2) and nitric oxide (NO) synthesis. For Group III PH, which is secondary to lung diseases, few recent clinical trials (ref 1) have demonstrated a benefit for the conventional therapies. Extracellular nucleotides, such as ATP, are involved in the modulation of the vascular tone via purinergic receptors present in the human pulmonary arteries (HPA) (ref 2). It has also been proven that the vasodilatation induced by acetylcholine (ACh) in vessels through muscarinic receptors has been associated with the release of NO and PGI_2 (ref 3).

Aims: To compare ATP- and ACh- induced relaxation in HPA derived from Group III PH and non-PH patients and the endothelium-derived mediators involved in these responses.

Methods: The relaxant effect of ATP and ACh was evaluated on the vascular tone of HPA derived from PH and non-PH patients by using an organ bath system. Organ bath supernatants were used for ELISA measurement of 6-keto-PGF_1alpha (a stable metabolite of PGI_2) after 30 min stimulation of HPA either with ACh 1 microM or ATP 0.1 mM.

Results: ATP and ACh produced endothelium-dependent relaxation of HPA and these effects were both PGI_2 and NO dependent. However, the relaxations induced by ATP were more potent. In HPA from Group III PH patients, ATP- and ACh- induced relaxation were dramatically attenuated.
In HPA derived from non-PH patients, ATP-induced relaxation was significantly inhibited when treated with either indomethacin or L-NOARG, and their combination had an additive inhibitory effect.
The measurements of 6-keto-PGF_1alpha could explain the different relaxations induced by ACh and ATP, will be presented.

Conclusions: Our results demonstrate that the endothelial dysfunction observed in PH vessels could involve PGI_2 and NO, in particular during ATP induced relaxations.

References: References: 1. PMID: 33440084; 2. PMID: 32037507; 3. PMID: 15172959

EFFECTS OF MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 (MPGES-1) INHIBITION ON RESISTANCE ARTERY TONE IN PATIENTS WITH END STAGE KIDNEY DISEASE

Julia Steinmetz-Spãh[1], SAMSUL AREFIN[2], Karin Larsson[1], Jabin Jahan[1], Neja Mudrovcic[2], Lars Wennberg[3], Peter Stenvinkel[2], Marina Korotkova[1], Karolina Kublickiene[2], Per-Johan Jakobsson[1]

1) Division of Rheumatology, Department of Medicine, Solna, Karolinska Institutet & Karolinska University Hospital, Stockholm, Sweden
2) Division of Renal Medicine, Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska University Hospital, Stockholm, Sweden
3) Division of Transplantation Surgery, Department of Clinical Science, Intervention and Technology, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden

A major concern with NSAIDs, selectively inhibiting cyclooxygenase2 (COX-2), is the increased risk for severe cardiovascular events, especially in patients suffering from chronic inflammation. The cardiovascular adverse effects of NSAIDS are widely ascribed to be caused by an imbalance in pro-thrombotic versus anti-thrombotic mediators resulting in thrombotic events, increased arterial blood pressure and heart failure. Inhibition of mPGES-1 the terminal synthase of PGE_2, resembles a novel approach for anti-inflammatory treatment leading besides the reduction of pro-inflammatory PGE_2 to a redirection of excess PGH_2 into the vasodilating PGI_2 pathway. The microvasculature is a central target organ for early manifestations of cardiovascular diseases. Therefore, a better understanding of the prostaglandin system and characterizing the effects of mPGES-1 inhibition in this vascular bed are of interest.

Here we studied the effects of mPGES-1 inhibition on constriction and relaxation of resistance arteries from patients with end stage kidney disease (ESKD) and controls using wire-myography in combination with immunological and mass-spectrometry based analyses.

Inhibition of mPGES-1 in arteries from ESKD patients and controls significantly reduced adrenergic vasoconstriction, which was not affected by the COX-2 inhibitors NS-398, Etoricoxib or the COX-1/COX-2 inhibitor Indomethacin. Correspondingly, a significant increase in

acetylcholine-induced dilatation and spared PGI_2 levels were observed for mPGES-1 inhibition only. Blockage of IP, EP4 or PPARÎ³ signaling did not restore the reduced constriction following mPGES-1 inhibition. Expression of mPGES-1, COX-1, PGIS and weak expression for COX-2 was found in ESKD and control arteries as well as receptor expression for PGE_2 (EP1-4), thromboxane (TP) and PGI_2 (IP).

In conclusion, our study demonstrates vasoactive effects of mPGES-1 inhibition and suggests that several pathways besides shunting to PGI_2 may be involved in the vasodilating effects following mPGES-1 inhibition in human microvasculature. The presented results motivate for further studies on mPGES-1 inhibition in diseases like Raynaud's phenomenon or myocardial infarction.

2nd Sponsored Session

PRO-RESOLVING LIPID MEDIATORS ARE NOVEL SIGNALS IN RESOLUTION AND TISSUE REGENERATION

CHARLES NICHOLAS SERHAN

Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

Specialized pro-resolving mediators (SPM) promote tissue regeneration, control infections and resolution of inflammation that will be reviewed in this presentation. These include resolvins, protectins, maresins and the newly identified conjugates in tissue regeneration (CTRs). We sought mechanisms of CTRs in activating primordial regeneration pathways in planaria (*Dugesia japonica*) using RNA-sequencing [PNAS. 2021;118(10): e2013374118]. The three CTRs (MCTR3, PCTR3 and RCTR3) share up-regulation of 175 known transcripts and 199 canonical pathways, including NF-κB pathways and an ortholog of human TRAF3. In human macrophages (MΦ) and mouse infections, CTRs regulate the TRAF3/IL-10 axis in enhancing phagocyte functions in resolution. CTR activation of TRAF3 signaling is a molecular component of both regeneration and resolution of infectious inflammation. Neutrophil extracellular traps (NETs) play a critical role in bacterial and viral infections, as in SARS-CoV-2 infections. We tested whether the recently identified 13-series resolvins (RvTs) regulate NET formation. Using microfluidic devices capturing NETs with human whole blood, Resolvin D2 and the RvTs each potently (RvT1-RvT4; 2.5 nM) reduce NETs [Blood. 2022; 139(8): 1222–1233]. These results provide evidence that resolvins reduce NETosis and enhance macrophage NET clearance.

The proposed 4S,5S-epoxy-resolvin intermediate in the biosynthesis of resolvin D3 and resolvin D4. Was prepared by total organic synthesis and we found that human neutrophils converted this synthetic intermediate to resolvin D3 and resolvin D4. M2 macrophages transformed this labile epoxide intermediate to resolvin D4 and a novel cysteinyl-resolvin that proved to be an isomer of RCTR1 without appreciable amounts of resolvin D3 (Shay et al PNAS e2116559118). M2 macrophages play key roles in the resolution of inflammation and wound healing. Human M2 macrophages also converted leukotriene A_4 to lipoxins. The novel cysteinyl-resolvin significantly accelerated tissue regeneration of surgically injured planaria. In a model of human granuloma formation, this cysteinyl- resolvin isomer significantly inhibited granuloma development by human peripheral blood leukocytes. These results provide evidence for human cell type-specific role of 4*S*,5*S*-epoxy-resolvin in the biosynthesis of resolvin D3 by human neutrophils, resolvin D4 by both human M2 macrophages and neutrophils, and a novel cysteinyl-resolvin positional isomer produced by M2 macrophages that processes potent activities in granuloma formation, resolution and tissue regeneration.

Session 1

INFLAMMATORY MACROPHAGE MEMORY IN NSAID-EXACERBATED RESPIRATORY DISEASE

Pascal Haimerl, Ulrike Bernhardt, Sonja Schindela, Fiona Henkel, Antonie Lechner, Ulrich Zissler, Xavier Pastor, Dominique Thomas, Alexander Cecil, Yan Ge, Mark Haid, Cornelia Prehn, Janina Tokarz, Matthias Heinig, Jerzy Adamski, Carsten Schmidt-Weber, Adam Chaker, JULIA ESSER-VON BIEREN

Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Zentrum München, 80802 Munich, Germany

Background - NSAID-exacerbated respiratory disease (N-ERD) is a chronic type-2 inflammatory condition, which is driven by an aberrant arachidonic acid (AA) metabolism. Macrophages are major producers of AA metabolites and subject to metabolic reprogramming, but they have been neglected in N-ERD.

Objective - We sought to elucidate a potential metabolic and epigenetic macrophage reprogramming in N-ERD.

Methods - Transcriptional, metabolic and lipid mediator profiles in macrophages from N-ERD patients and healthy controls were assessed by RNA sequencing, Seahorse assays and LC-MS/MS. Metabolites in nasal lining fluid (NLF), sputum and plasma from N-ERD patients (n=15) and healthy individuals (n=10) were quantified by targeted metabolomics analyses. Genome-wide methylomics were deployed to define epigenetic mechanisms of macrophage reprogramming in N-ERD.

Results - We show that N-ERD monocytes/ macrophages exhibit an overall reduction in DNA methylation, aberrant metabolic profiles and an increased expression of chemokines, indicative of a persistent pro-inflammatory activation. Differentially methylated regions in N-ERD macrophages included genes involved in chemokine signaling and acylcarnitine metabolism. Acylcarnitines were increased in macrophages, sputum, NLF and plasma of N-ERD patients. Upon inflammatory challenge, N-ERD macrophages produced increased levels of acylcarnitines, pro-inflammatory AA metabolites, cytokines and chemokines as compared to healthy macrophages.

Conclusion - Together, our findings decipher a pro-inflammatory metabolic and epigenetic reprogramming of macrophages in patients suffering type-2 inflammation.

Session 2

ROLES OF AA AND DHA IN VIVO-FROM MEDIATORS TO MEMBRANE

TAKAO SHIMIZU

University of Tokyo, National Center for Global Health and Medicine, Japan

Arachidonic acid (AA) and docosahexaenoic acid (DHA) are important precursors of lipid mediators involved in a variety of biological functions including inflammation and its resolution. These PUFAs are mostly esterified at sn-2 position of glycerophospholipids, and free fatty acids are released by mainly Ca-dependent phospholipase A2s. The questions are the mechanisms of how individual PUFAs are accumulated at sn-2 of glycerophospholipids, and in vivo functions of PUFAs in membrane other than mediator precursors roles (1-3).

To answer these questions, we started to screen genes in the biosynthesis of glycerophospholipids with a variety fatty acid combinations (more than 1,000 species). We isolated 9 genes (6 in AGPAT and 3 in MBOAT family) with different donor (acyl-CoAs) and lysophospholipid acceptor specificities. Today, we present data focusing on 2 enzymes, LPCAT3 (lysophosphatidylcholine acyltransferase 3, LPLAT12 in new nomenclature) and LPAAT3 (lysophosphatidic acid acyltransferase 3, AGPAT3, LPLAT3), crucial players to incorporate AA and DHA to phospholipid membranes, respectively (3).

LPCAT3-null mice are born normally but postnatally lethal. This is mostly due to malnutrition caused by the damage of intestine epithelium. Both intestinal cells and hepatocytes showed fatty degeneration containing excess triglycerides droplets ,while serum chylomicron and VLDL are markedly reduced. Together with biochemical analyses, we propose that AA-rich membrane in ER is critical for production of lipoproteins by uptake of cytosolic triglyceride with the help of microsomal triglyceride transfer protein (MTTP). LPAAT3 mice showed male infertility and light-independent blindness, caused by deficiency of DHA-phospholipids in Sertoli cells/sperm and photoreceptor cells, respectively. Noteworthy, while DHA is decreased in membrane phospholipids, AA appears increased as compensation, showing that total PUFA proportion is monitored and unchanged. Although detailed mechanism remains clarified, either docosanoids, DHA binding proteins or DHA-rich milieu would explain (4-6).

Ref.

1. Shimizu, T. (2009) Ann. Rev. Pharmacol. Toxicol. 49, 123
2. Harayama, T. and Shimizu, T. (2020) J. Lipid. Res. 61, 1150
3. Valentine, W. et al. (2022). J. Biol. Chem. 298, 101470
4. Iizuka-Hishikawa, Y. et al. (2017) J. Biol. Chem, 292, 12065
5. Shindou, H. et al. (2017) J. Biol Chem. 292, 10586
6. Hishikawa, D. et al. (2020) iScience 22:23, 101495

EXCESSIVE DIETARY LINOLEIC ACID PROMOTES PLASMA ACCUMULATION OF PRONOCICEPTIVE LIPID MEDIATORS AND THERMAL NOCICEPTIVE HYPERSENSITIVITY

CHRISTIAN A. REYNOLDS, Toni Azar, Nada Birkic, Krishna Rao Maddipati, and Zeljka Minic Department of Emergency Medicine, Wayne State University School of Medicine, Detroit, Michigan, USA

Various fatty acyl lipid mediators are derived from dietary polyunsaturated fatty acids (PUFAs) and modulate nociception. The modern diet is rich in linoleic acid, which may present a risk factor for developing pain conditions. Although recommendations about fatty acid intake exist for some diseases (e.g. cardiovascular disease), the role of dietary fatty acids in promoting pain disorders is not completely understood. Here we report that dietary linoleic acid content is associated with plasma accumulation of pronociceptive lipid mediators. From the time of weaning, male and female rats were randomized to receive one of two modified AIN-76A rodent diets each containing 5.1% fat. The standard corn oil was replaced with a custom triglyceride blend rich in either linolic acid (LA, 18:2n-6) or oleic acid (OA, 18:1n-9). At nine weeks of age, paw-withdrawal latencies were assessed in response to thermal stimulation and plasma was collected for fatty acyl lipidomic analysis. Thermal nociceptive hypersensitivity was observed in animals exposed to a LA-rich diet. In general, rats maintained on the LA-rich diet displayed greater plasma accumulation of fatty acyl lipid mediators derived from LA and arachidonic acid (AA), while accumulation of fatty acyl lipid mediators derived from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was greater in rats maintained on the OA-rich diet. Univariate analysis detected 28 fatty acyl lipid mediators with a p-value < 0.05 and a log2fold change >1.5. Many of the fatty acyl lipid mediators that were elevated in animals maintained on the LA-rich diet are known to interact with transient receptor potential (TRP) channels. Our findings provide mechanistic insights into exaggerated nociceptive hypersensitivity associated with excessive dietary linoleic acid intake and highlight potential biomarkers for pain risk stratification.

MOLECULAR REGULATION OF ENDOTHELIAL AND VASCULAR DYSFUNCTION BY PUFA-DERIVED LACTONE MEDIATORS

Bar-Sheshet Michal, Ertracht Offir, Reuveni Tal, Atar Shaul, SZUCHMAN-SAPIR ANDREA

MIGAL Research Institute, Tarshish 2, Kiryat-Shmoan, Israel

Microvascular dysfunction is a known contributor to cardiovascular-related diseases. Under these conditions, nitric oxide-mediated endothelial dilation loss results in an alternative pathway regulating vascular tone by endothelial-derived hyperpolarizing factors (EDHFs). Previously, we showed that lactone metabolite derived from the arachidonic (AA-L) and eicosapentaenoic (EPA-L) acid metabolism induces endothelial-dependent vasodilation in isolated human microvessels.

To further investigate and characterize the molecular mechanisms by which these metabolites mediate endothelial hyperpolarization in microvascular dysfunction in vivo, 5/6Nx hypertensive rats were intravenously administrated with EPA-L (3mg/Kg) for five days. Blood pressure (BP), blood and urine chemistry, and kidney function were analyzed. A pressure myograph detected EPA-L-induced vascular dilation of microvessels isolated from hypertensive (HTN) versus normotensive volunteers. The signaling mechanism was investigated by potassium efflux with antagonists for GPCRs and the PLC-IP3 pathway in endothelial cells. Results: EPA-L significantly reduced BP and heart rate of 5/6Nx rats without affecting kidney function. EPA-L restored vessel relaxation response in 5/6Nx rat mesenteric arterioles. TRAM-34 and apamin significantly reduced the EPA-L relaxation. EPA-L initiates dose-dependent potassium efflux in endothelial cells, which was impaired by inhibiting the GPCR βγ subunit, PLC, IP3 receptor, and the calcium-dependent potassium channels IK and SK. In addition, gene expression analysis was performed using scRNA sequencing for the mesenteric arteries. We conclude that PUFA- derived lactones are novel EDHFs that reduce blood pressure by initiating microvascular dilation. The endothelial hyperpolarization mechanism depends on the GPCR-PLC-IP3 signaling pathway, activating calcium-derived potassium efflux.

PERFORMANCE AND LIMITATION OF STATE-OF-THE-ART QUANTITATIVE ANALYSIS FOR SPECIALIZED PRO- RESOLVING MEDIATORS

NILS HELGE SCHEBB

Chair of Food Chemistry, University of Wuppertal, Gausstraße 20, 42119 Wuppertal, Germany

In the last decades, specialized pro-resolving mediators (SPMs) have gained increasing attention due to their putative role in the resolution of inflammation. The required sensitivity and selectivity for oxylipin analysis including SPMs in biological samples can only be reached using chromatography coupled to mass spectrometry (MS). To date, only liquid chromatography coupled via electrospray ionization (ESI) to tandem MS – with almost identical instrumentation – is frequently used for quantitative SPM analysis. However, the reported lower limit of quantification (LLOQ) – which is the lowest concentration that can be reliably quantified in a sample – varies dramatically. In our hands, the state-of-the-art instruments operated as described above reach a sensitivity of 0.5 to 5 pg on column for SPMs. In fact, this is the same range as for other oxylipins which is consistent with their chemical structural similarity. However, several groups in the field of SPM research report a sensitivity which is 5-10-fold better (0.05 to 5 pg on column) using another definition of the sensitivity, i.e. the LLOQ.

In the talk, the LC-MS-based quantification and its optimization for the detection of oxylipins including SPMs is briefly summarized and the common LLOQ definition e.g. established by the US Food and Drug Administration and the European Medicines Agency is explained.

Based on that it is shown that the methods using an alternative LLOQ definition for SPM detection cannot be considered to allow a reliable determination of their concentration. Taking the reported SPM concentrations in most biological samples and a sound LLOQ into account, it can be concluded that SPM concentrations are too low to be detected and quantified reliably with state-of-the-art instrumentation.

Schebb et al., Frontiers in Pharmacology, 13, 838782, doi 10.3389/fphar.2022

Session 3

MOLECULAR DISSECTION OF CERAMIDE-INDUCED APOPTOSIS USING LIGHT-DRIVEN LIPID PROBES

JOOST C. M. HOLTHUIS

Molecular Cell Biology Division, Department of Biology/Chemistry & Center for Cellular Nanoanalytics Osnabrueck, University of Osnabrueck, 49076 Osnabrueck, Germany

Ceramides attract wide attention as tumor suppressor lipids that can act directly on mitochondria to trigger Bax-mediated cell death. While ceramide engagement in mitochondrial apoptosis is clinically relevant, molecular details of the underlying mechanism are largely unknown. A chemical screen for ceramide binding proteins combined with computer simulations and functional studies in cancer cells previously led us to identify the voltage-dependent anion channel VDAC2 as critical effector of ceramide-mediated apoptosis. VDAC residues involved in ceramide binding are also required for mobilizing hexokinase type-I to mitochondria, a potential checkpoint in apoptosis.

Collectively, our data support a model in which ceramides function as modulators of VDAC-based platforms to control mitochondrial recruitment of pro- and anti-apoptotic machinery. The central aim of the current project is to challenge fundamental aspects of this model by exploiting switchable ceramide transfer proteins and mitochondrial-specific release of photocaged ceramides in combination with live cell imaging and functional studies. Understanding the molecular principles by which ceramides commit cells to death may facilitate the development of novel strategies to enhance their anti-tumor potential for therapeutic treatment.

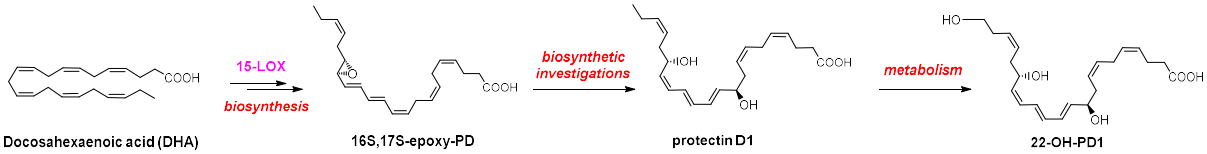
ELUCIDATION OF THE BIOSYNTHETIC PATHWAYS OF THE PROTECTINS

TROND VIDAR HANSEN

Department of Pharmacy, University of Oslo, PO Box 1068, N-0316, Oslo, Norway. t.v.hansen@farmasi.uio.no

Several ω-3 and the ω-6 polyunsaturated fatty acids are enzymatically converted during inflammation-resolution processes into various families of specialized pro-resolving mediators (SPMs).[1] These distinct biosynthetic pathways result in the formation of unique families of SPMs coined lipoxins, D-, E- and T-series of resolvins, maresins, protectins and peptide conjugates. The SPMs promote clearance of bacteria and apoptotic cells, down regulate pro-inflammatory mediator production and stimulate the resolution of inflammation.[1]

In this presentation, results from biosynthetic studies and biological evaluations of the protectin family of SPMs will be presented.[2]



1. Serhan, C. N. *Nature* **2014**, *510*, 92.
2. See the LIPCHEM web-site at www.mn.uio.no/farmasi/english/research/groups/lipchem/ for more information and recent publications.

TRANSCELLULAR METABOLISM IN BINARY CELL INCUBATIONS DIFFERENTIALLY AFFECTS SPM BIOSYNTHESIS

MARKUS WERNER and Lena Hegner, Oliver Werz

Institute of Pharmacy, Philosophenweg 14, 07743 Jena, Germany

Lipid mediators (LM) play pivotal roles in inflammation, from initiation to resolution. While prostaglandins and leukotrienes are key players in the acute phase of inflammation, the specialized pro-resolving mediators (SPM) are involved in the resolution phase. SPM formation typically relies on two sequential oxygenation steps of polyunsaturated fatty acids, involving 5-lipoxygenase (5-LO) and different types of 12- and 15-lipoxygenating paralogues (e.g., 15-LO1, 15-LO2, 12-LO). Specific cell types express primarily one or two major LM-producing enzymes, for example, 5-LO in neutrophils, 12-LO in platelets and 15-LO in M2 macrophages. Potentially, during an immune response such limited compartmentalization of LM production by specific enzymes in one cell type can be overcome and several cell types might cooperatively produce substantial amounts of SPM. Following this principle, coincubations of neutrophils (expressing 5-LO) with platelets (expressing 12-LO) were already confirmed as potential source for strong lipoxin formation. Along these lines, we here systematically studied different coincubations of LO-positive peripheral blood cells (platelets, neutrophils, and monocytes) as well as different macrophage subtypes (M0, M2c and M2a), which may physiologically appear during the transition of inflammation to its resolution trying to detect extraordinary strong sources of SPM formation. We first systematically compared these cells in single incubations regarding their potential to form LM using a total protein normalization approach. M2a macrophages are clearly the most potent SPM producers. Notably, the LM profiles are strongly dependent on the stimulus: in M2a macrophages the resolvin D5/LTB_4 ratio is 1.3 for stimulation by exotoxin but only 0.1 upon stimulation by ionophore A23187. We confirmed strong synergistic lipoxin formation in neutrophil-platelets cocultures upon stimulation by bacterial exotoxins. Similar observations were made for monocytes-platelet cocultures and to a lesser extend for neutrophil-monocytes cocultures. Intriguingly, coincubations of 15-LO-1-positive M2a with neutrophils strongly abolished resolvin D5, resolvin E4 and 5,15-diHETE formation versus M2a in single incubations, while coculture of M2c (high 15-LO-2 expression) with neutrophils amplified SPM biosynthesis. Together, our data show that LM profiles of immunological active cells strongly differ depending on the stimulus and can vary remarkably in more complex but biological relevant coincubations versus single cell cultures.

CHEMICAL PROBES FOR TARGETS IN LIPID SIGNALLING

EUGEN PROSCHAK

Goethe-University Frankfurt, Max-von-Laue Str. 9 D-60438 Frankfurt Germany

Signalling through lipid modulators plays a major role in physiological and pathophysiological processes. Many targets involved in biosynthesis, signal transduction, and degradation of lipid modulators are in the focus of drug discovery. High-quality chemical probes and pharmacological tools are needed for target validation.1 Here, we present the development of two chemical probes for emerging targets.

G2A (GPR132) is a proton sensing GPCR, which is also modulated by lipid modulators. Different endogenous ligands including 9-HODE and N-palmitoylglycine activate G2A, however, no synthetic agonists suitable for target validation have been reported so far. We performed HTS of 20,000 compounds using stably transfected CHO K1 cells to identify synthetic agonists. After optimizing the validated hit, we ended up with a potent and selective chemical probe, which allowed us to investigate the role of G2A in the context of leukemia.

The emerging pharmacological target soluble epoxide hydrolase (sEH) is a bifunctional enzyme exhibiting two different catalytic activities that are located in two distinct domains. Although the physiological role of the C-terminal hydrolase domain is well-investigated, little is known about its phosphatase activity, located in the N-terminal phosphatase domain of sEH (sEH-P). We report on the discovery and optimization of the first inhibitor of human and rat sEH-P that is applicable in vivo.

X-ray structure analysis of the sEH phosphatase domain complexed with an inhibitor provides insights in the molecular basis of small-molecule sEH-P inhibition and helps to rationalize the SAR. Our tool compound has an excellent pharmacokinetic and pharmacodynamic profile in rats and enables the investigation of the physiological and pathophysiological role of sEH-P in vivo.

FUNCTIONAL CHARACTERISATION OF MAST CELL ACTIVATION BY IGE AND HYPEROSMOLARLARITY IN ISOLATED HUMAN SMALL AIRWAYS

JESPER SÄFHOLM, Sven-Erik Dahlén, and Mikael Adner

Karolinska Institutet, Biomedicum, Q5B, Solnavägen 9, 17177 Stockholm Abstract:

Introduction: In asthmatics, acute mast cell induced bronchoconstriction can be initiated by allergens (IgE crosslinking) and exercise (local increase of osmolarity). The aim of this study was to characterise the role of prostaglandins in the reactions by performing pharmacomechanistic studies using isolated human bronchi.

Methods: Human small airways (inner diameter of 0.5-2 mm) were isolated from macroscopically healthy human lung tissue specimens obtained from patients undergoing lobectomies (n=16). The segments were incubated overnight and mounted in tissue organ baths to measure smooth muscle contractions evoked by challenge with either hyperosmolar mannitol or a monoclonal anti-human IgE antibody (anti-IgE).

Results: In control segments, exposure to hyperosmolar mannitol (850 mOsm) caused an acute contraction (Emax:49±5%) reaching maximum within 10 minutes. Anti-IgE (5 µg/mL) also induced a contractile response within the same timeframe that was more powerful (Emax:89±8%). Using a combination of receptor antagonists blocking histamine H1 and cysteinyl-leukotriene cysLT1 receptors reduced both the hyperosmolar and anti-IgE induced contractions by 20% and 52%, respectively. Furthermore, by also adding a thromboxane TP receptor antagonist, both the hyperosmolar and anti-IgE induced contractions were completely prevented. Likewise, global inhibition of the cyclooxygenase (COX) enzymes and inhibition of COX-1, in combination with the H1 and cysLT1 receptor antagonism, completely prevented the bronchoconstriction for both triggers. In contrast, this effect was not observed after COX-2 inhibition, which instead enhanced the bronchoconstriction for anti-IgE by 30%, but not for mannitol.

Conclusion: It was confirmed that mast cell dependent contractions of human bronchi are mediated by histamine, cysteinyl-leukotrienes and COX-1 generated contractile prostanoids acting on the TP receptor. The relative contribution of the TP receptor was greater during hyperosmolarity than for anti-IgE challenge. Finally, the potentiation of the response to anti-IgE by COX-2 inhibition is most likely due to removal of bronchoprotective PGE_2.

Session 4

SPECIFICITY OF PHOSPHOLIPASE A2S REGULATE WHICH DOWNSTREAM PUFA-DERIVED MEDIATORS ARE PRODUCED

EDWARD A. DENNIS, Daiki Hayashi, and Varnavas D. Mouchlis

Department of Chemistry and Biochemistry and Department of Pharmacology, School of Medicine, University of California at San Diego, La Jolla, California, 92093 USA

Lipid mediators play critical roles in metabolic syndrome and inflammation, which encompass the major diseases of our times, and over 47,000 distinct molecular species of lipids have now been identified by the LIPID MAPS Consortium (www.lipidmaps.org). Our laboratory demonstrated that membranes interact allosterically with phospholipase A2 (PLA2) enzymes to regulate cell signaling, membrane remodeling and lipid mediator production by their specificity at the molecular species level. We have recently developed substrate lipidomics using UPLC/MS coupled with molecular dynamics simulations to reveal enzyme specificity linked to highly specific hydrophobic binding sites for the specific sn-2 fatty acyl chains released in membrane phospholipid substrates [Mouchlis et al (2018) J Am Chem Soc]. Stereospecific inhibitors for optimal substrates have been designed for the specific hydrophobic sites [Mouchlis et al (2019) J Med Chem]. We have now discovered unexpected head-group and acyl chain specificity for each of the 4 major types of human PLA2's that explains the observed specificity at a new atomic level. A unique hydrophobic binding site — and not each enzyme's catalytic residues or polar head-group binding site — dominates each enzyme's specificity. Each PLA2 shows unique specificity for its required sn-2 fatty acyl with cPLA2 favoring pro-inflammatory omega-6 arachidonic acid and sPLA2 favoring anti-inflammatory fish oil omega-3 DHA [Hayashi et al (2021) J Lipid Res]; others like iPLA2 favor omega-3 EPA and membrane remodeling linolenic acid, while LpPLA2/PAFAH favors oxidized fatty acids in LDL [Mouchlis et al (2022) Proc Natl Acad Sci USA]. Furthermore, plasmalogens or alkyl ethers in the sn-1 acyl chain can affect this selectivity [Hayashi et al (2022) Biochim Biophys Acta, Mol Bio Lipids]. We found that each PLA2 releases a specific fatty acid after the enzyme associates allosterically with membranes and extracts a single phospholipid substrate into its catalytic site [Review: Dennis (2022) J Biol Chem]. We can now correlate PLA2 specificity and inhibition potency with molecular structure and physiological function using a novel lipidomics platform that provides a paradigm at the molecular species specificity level for enzyme specificity in lipid metabolism and the selectivity for pro-inflammatory or anti-inflammatory lipid mediator production in macrophages in vivo.

RESOLVIN D1 AND D2 REDUCE INFLAMMATORY RESPONSE TO SARS-COV-2

ANTONIO RECCHIUTI, Domenico Mattoscio, Sara Patruno, Giulia Ferri, Matteo Mucci, Romina Pecce, Antonella Pomilio, Mario Romano

Università “G. d'Annunzio”, University of Chieti Department of Medical, Oral, and Biotechnology Science, 66100 Chieti, Italy

The COVID-19 pandemic has made sparkly clear that resolution of inflammation is essential avoid further damage due to an excessive host response and severe symptoms even in fully vaccinated people. This is mostly important in people with cystic fibrosis (CF) who have lung disease, chronic inflammation, and persistent respiratory infection. Given the important pro-resolutive functions of resolvin (Rv) D1 and D2, here we determined their bioactions on CF and non-CF macrophages (MF) following SARS-CoV-2 stimulus.

In CF and non-CF MF stimulated (3 h) with the SARS-CoV-2 spike protein 1 (S1), RvD1 and RvD2 (each 10 nM) significantly stopped the release of chemokines interleukin (IL)-8, monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein (MIP)1 beta that are essential for driving further leukocyte recruitment during SARS-CoV-2 infection. RvD1 and RvD2 also blunted tumor necrosis factor (TNF) alpha and IL-6 secretion that were increased by S1 selectively in non CF MF. These counterbalancing actions of RvD1 and RvD2 were also evident following a therapeutic administration of RvD1 and RvD2 to MF after stimulation with S1.

Mechanistically, we found that RvD1 and RvD2 both restored the expression of miR-16 and miR-29a that were downregulated by S1 in CF and non-CF MF and stop NF-kB signaling and MF hyperactivation.

RvD1 and RvD2 also significantly activated bacterial clearing phagocytosis of CF and non-CF MF during *P. aeruginosa* and S1 stimulation.

Together, these findings provide the first evidence regulatory activities of resolvins on inflammation and anti-microbial responses of MΦ to SARS-CoV-2, highlighting the essential roles of SPM in the regulating human immunity against this virus.

SMALL EXTRACELLULAR VESICLE-DERIVED MIR-574-5P REGULATES PGE2-BIOSYNTHESIS VIA TLR7/8 IN LUNG CANCER

Julia Donzelli, Eva Proestler, Anna Riedel, Sheila Nevermann, Brigitte Hertel, Andreas Guenther, Stefan Gattenlöhner, Rajkumar Savai, Karin Larsson, MEIKE J. SAUL

Meike J. Saul Department of Biology Technische Universität Darmstadt, Schnittspahnstraße 10, 64287 Darmstadt/Germany

Intercellular communication plays an essential role in lung cancer (LC). One of the major players in cell-cell-communication is small extracellular vesicles (sEV). SEV trigger various biological responses by transporting cellular cargo to target cells. One essential sEV component are microRNAs (miRs), whose transport has recently attracted increasing research interest. We report that prostaglandin E2 (PGE2), a key inflammatory lipid mediator, specifically induces the sorting ofmiR-574-5p in sEV of A549 and 2106T cells. We found that sEV-derived miR-574-5p activates Toll-like receptors (TLR) 7/8, thereby decreasing PGE2-levels. In contrast, intracellular miR-574-5p induces PGE2-biosynthesis. Consequently, the combination of intracellular and sEV-derived miR-574-5p controls PGE2-levels via a feedback loop. This was only observed in adeno- but not in squamous cell carcinoma, indicating a cell-specific response to sEV-derived miRs, which might be due to unique tetraspanin compositions. Hence, we describe a novel function of miR-574-5p unique to adenocarcinoma. Intracellular miR-574-5p induces PGE2 and thus the secretion of sEV-derived miR-574-5p, which in turn decreases PGE2-biosynthesis in recipient cells.

Session 5

CYCLOOXYGENASE-2 AND HEART FAILURE WITH PRESERVED EJECTION FRACTION.

EMANUELA RICCIOTTI, Philip G. Haines, Manu Beerens, Uri Kartoun, Soon-Yew Tang, Hu Meng Nicholas F. Lahens, Tao Wang, Stanley Y. Shaw, Calum A. MacRae and Garret A. FitzGerald.

3400 Civic Center Boulevard, Philadelphia PA 19104, USA

Nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the activity of cyclooxygenases (COX)-1 and COX-2, are used to relieve pain and inflammation. Their use is associated with an increased risk of cardiovascular events, mainly atherothrombotic events and heart failure (HF). As well proven, atherothrombotic events are attributable to loss of cardioprotective

COX-2 derived prostaglandins with unrestrained platelet COX-1-derived thromboxane. Less is known about HF and it is thought that it might develop through a COX-2 dependent hazard, unrelated to variable COX-1 dependent platelet inhibition, that causes cardiovascular remodeling and impairment of renal function.

Here we investigated the type of HF, with reduced or preserved ejection fraction (HFpEF), that is associated with COX-2 disruption, its interaction with the syndrome's comorbidities (i.e. age and sex), and the underling mechanisms using a multi-species approach.

In larval zebrafish, pharmacological inhibition of COX-2 by celecoxib caused a modest but significant reduction in heart rate and diastolic function, while the ejection fraction (EF) remained preserved.

Furthermore, celecoxib caused a significant reduction in ventricular diastolic Ca^{2+} and an increase in ventricular Ca^{2+} transient amplitude, as seen in HFpEF.

Postnatal deletion of Cox-2, as well as pharmacological inhibition of COX-2, did not impair cardiac function in adult male and female mice. In contrast, elderly male Cox-2 KOs presented increased vascular stiffness and elderly female Cox-2 KOs exhibited diastolic dysfunction compared to age- matched control mice. The EF was preserved in all groups of mice studied.

The interrogation of the Harvard-Partners clinical EMR database of 314,000 subjects, using a multinomial multivariable regression approach, revealed an increased odds for HFpEF when subjects were exposed to COX-2 selective vs nonselective NSAIDs.

In conclusion, deletion or inhibition of COX-2 results in an HFpEF phenotype in zebrafish, mice and humans.

DHA, RVD1, RVD5 AND MAR1 REDUCE HUMAN CORONARY ARTERIES CONTRACTIONS INDUCED BY PGE2

BOUHADOUN A, Manikpurage HD, Dubourdeau M, Urbach V, Longrois D and Norel X

CHU X. Bichat, 46 rue Henri Huchard 75018 Paris, France

Background: Specialized pro-resolving lipid mediators (SPM) are known to reduce the progression of atherosclerosis[1]. Results of our group showed that prostaglandin E_2 (PGE_2) synthesis is increased in human coronary arteries (HCA) with atherosclerosis and is responsible for their vasoconstrictions[2]. Beneficial effects of omega-3 polyunsaturated fatty acids have been shown in the regulation of vascular tone in patients with coronary artery disease[3].

Aims: In our study the effects of different SPM [eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), RvD1, RvD5 and MaR1] have been determined on the vascular tone of isolated HCA. We also report the HCA cellular expression of their respective receptors: FPR2/ALX, GPR32, and LGR6 measured by immunofluorescence.

Methods: HCA were dissected from patient's hearts obtained after transplantation. Small rings of HCA were incubated during 18 hours with or without one of the cited SPM. Rings were set-up in an organ bath system and Concentration-response curve (CRC) experiments using vasoconstrictors, PGE_2 (1 nM-10 µM) or U46619 (TxA2 analogue, 0.1 nM-1 µM), were realized.

Results: Reduced PGE_2-CRC (20-30%) were measured only in HCA rings incubated with DHA (100 µM, n=5), RvD1, RvD5 or MaR1 (100 nM, n=12), while treatment with EPA (100 µM, n=6) showed no effect. In addition, both EPA and DHA (n=10-11) had no effect on U46619-CRC. Pre-incubation with indomethacin, a cyclooxygenase inhibitor (1.7 µM, n=6-7) during 30 minutes didn't modify subsequent PGE_2-CRC. Interestingly, incubation with L-NOARG, an inhibitor of NO-synthase (0.1 mM, n=7) reduced the PGE_2 contractions induced with 0.1 µM and 1 µM, only in the RvD1 treated rings.

Finally, our immunofluorescence results (n=3) showed the presence of FPR2/ALX, GPR32 and LGR6 receptors in HCA endothelial and smooth muscle cells.

Conclusions: DHA and some of its derived metabolites (RvD1, RvD5 and MaR1) decrease PGE_2- induced contraction in HCA. They are probably mediated through their respective receptors detected in the vascular wall of HCA and are independent from prostanoids pathways. Our results suggest these SPM as a therapeutic approach to reduce coronary artery spasm.

1) Fredman et al., Nat Commun, 2016; 2) Ozen et al., BPS e-journal, 2015; 3) Daci et al., Eur J Pharm Sci, 2020.

IMMUNOMODULATION BY INTRAVENOUS OMEGA-3 FATTY ACID TREATMENT IN OLDER SUBJECTS HOSPITALIZED FOR COVID-19: A SINGLE-BLIND RANDOMIZED CONTROLLED TRIAL

Hildur Arnardottir[1]*, SVEN-CHRISTIAN PAWELZIK[1]*, Philip Sarajlic[1], Alessandro Quaranta[2], Johan Kolmert[2,3], Dorota Religa[4], Craig E. Wheelock[2,5], Magnus Bäck[1]

** Shared first author*

1) Department of Medicine, Karolinska Institutet, Theme Heart, Vessels, and Neuro, Karolinska University Hospital, Stockholm, Sweden

2) Division of Physiological Chemistry 2, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.

3) The Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

4) Department of Neurobiology, Karolinska Institutet and Theme Ageing, Karolinska University Hospital, Stockholm, Sweden

5) Department of Respiratory Medicine and Allergy, Karolinska University Hospital, Stockholm, Sweden.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19) with respiratory distress and systemic hyperinflammation. The primary objective of this single-blind randomized controlled proof-of-concept clinical trial was to establish the effects of intravenous (i.v.) omega-3 (n-3) polyunsaturated fatty acid (PUFA) treatment compared to placebo on inflammatory markers in COVID-19, represented by leukocytes as well as inflammatory protein and lipid mediators. Here we also present an exploratory analysis of the mechanisms of action to elucidate the potential to resolve the COVID-19 hyperinflammation through interfering with lipid mediators.

Inclusion criteria were COVID-19 diagnosis and clinical status requiring hospitalization. Randomization was 1:1 to a once daily i.v. infusion (2 ml/kg) of either n-3 PUFA emulsion containing 10 g of fish oil per 100 ml or placebo (NaCl) for 5 days. Results from 22 older subjects (mean age 81±6.1 years) were analyzed. The neutrophil-to-lymphocyte-ratio was significantly decreased after n-3 PUFA administration. Liquid chromatography mass spectrometry (LC-MS/MS)-based lipid metabolite analysis established increased proresolving lipid mediator precursor levels and decreased formation of leukotoxin and isoleukotoxin diols by n-3 PUFA treatment. The mechanistic exploration revealed decreased immunothrombosis and preserved interferon-response. Finally, n-3 PUFA treatment may serve to limit cortisone-induced immunosuppression, including preserving leukocyte phagocytic capacity. In conclusion, i.v. n-3 PUFA administration was safe and feasible during hospitalization of multimorbid older subjects for COVID-19. The results identified n-3 PUFA treatment mediated lipid signature of increased proresolving precursor levels and decreased leukotoxin diols in parallel to beneficial immune responses. EudraCT: 2020-002293-28; clinicaltrials.gov: NCT04647604.

Young Investigator Symposium

MACROPHAGES ACQUIRE A TNF-DEPENDENT INFLAMMATORY MEMORY IN ALLERGIC ASTHMA

Antonie Lechner[1], FRANZISKA HARTUNG[1], Fiona Henkel[1], Sina Bohnacker[1], Francesca Alessandrini[1], Ekaterina O. Gubernatorova[2], Marina S. Drutskaya[2], Carlo Angioni[3], Yannick Schreiber[4], Pascal Haimerl[1], Yan Ge[5], Dominique Thomas[3], Caspar Ohnmacht[1], Sergei A. Nedospasov[2], Peter J. Murray[6], Adam M. Chaker[1,7], Carsten B. Schmidt-Weber[1,8], Julia Esser-von Bieren[1]

1) Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, 80802 Munich, Germany
2) Center for Precision Genome Editing and Genetic Technologies for Biomedicine, Engelhardt Institute of Molecular Biology, Moscow, 119991 and Sirius University of Science and Technology, Sochi, 354349, Russia
3) Institute of Clinical Pharmacology, Goethe-University Frankfurt, 60590 Frankfurt am Main, Germany
4) Fraunhofer Institute for Translational Medicine and Pharmacology (ITMP), 60596 Frankfurt am Main, Germany
5) Immunobiology, Hospital Carl Gustav Carus, University of Dresden, Dresden Germany
6) Max Planck Institute of Biochemistry, 82152 Martinsried, Germany
7) Department of Otorhinolaryngology and Head and Neck Surgery, TUM Medical School, Technical University of Munich, 81675 Munich, Germany
8) Member of the German Center of Lung Research (DZL)

Infectious agents can reprogram or train macrophages and their progenitors to respond more readily to subsequent insults. However, whether such an inflammatory memory exists in type-2 inflammatory conditions such as allergic asthma was not known. Therefore, we aim to elucidate the role of trained immunity, especially of macrophages, in allergic airway inflammation.

We used clinical sampling of house dust mite (HDM)-allergic patients, HDM-induced allergic airway inflammation (AAI) in mice and an in vitro training set-up to analyze persistent changes in macrophage eicosanoid-, cytokine- and chemokine production as well as underlying metabolic and epigenetic mechanisms. Transcriptional profiles of patient-derived and in vitro trained macrophages were assessed by RNA sequencing and LC-MS/MS analysis, respectively.

We found that macrophages differentiated from bone marrow- or blood monocyte-progenitors of HDM-allergic mice or asthma patients showed inflammatory transcriptional reprogramming and excessive mediator (TNF, CCL17, leukotriene, IL-6) responses upon stimulation. Macrophages from HDM-allergic mice initially exhibited a type-2 imprint, which shifted towards a classical inflammatory training over time. In vitro HDM-induced macrophage training was mediated by a formyl-peptide receptor 2 (FPR2)-TNF-axis, resulting in an M2-like macrophage phenotype with high CCL17 production. TNF blockade by etanercept or genetic ablation of Tnf in myeloid cells prevented the inflammatory imprinting of bone marrow-derived macrophages from HDM-allergic mice.

Our findings suggest that allergen-triggered inflammation drives a TNF-dependent innate memory, which may perpetuate and exacerbate chronic type-2 airway inflammation and thus represents a potential target for asthma therapy.

METABOLOLIPIDOMIC AND PROTEOMIC PROFILING REVEALS ABERRANT MACROPHAGE POLARIZATION AND INTERRELATED LIPID MEDIATOR FORMATION DURING AGING

PATRICK SCHÄDEL[1], Anna Czapka[1,2], Nadja Gebert[3,4], Alessandro Ori[4] and Oliver Werz[1]

1) Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University Jena, Jena, Germany

2) Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute (HKI), Jena, Germany

3) ProteiQ Biosciences GmbH, Potsdam, Germany

4) Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena, Germany

Macrophages are major regulators of the innate immune response to infection or trauma and orchestrate the initiation, perpetuation and resolution of inflammation through the release of immunomodulatory mediators. A key characteristic of these phagocytes is their ability to adapt to the surrounding microenvironment through polarization towards a pro-inflammatory (M1) or pro- resolving phenotype (M2) and to release prostaglandins (PG) and leukotrienes (LT) or specialized pro- resolving mediators (SPM), respectively. These processes are vital for tissue-resident macrophages (TRMs) such as peritoneal macrophages (PM) to maintain tissue homeostasis and successfully combat invading pathogens. However, aging is known to markedly impact the effectiveness of the innate immune system and to increase the susceptibility of elderly individuals towards infection. Thus far, the influence of aging on the function of TRMs and the subsequent impact on their ability to exert distinct roles during inflammation remain elusive. Here, we provide comprehensive insights into age-associated aberrations of murine PM activation and polarization with concomitant alterations to the metabololipidome during bacterial challenge. We found that aging reduces the expression of both cyclooxygenase-1 and 5-lipoxygenase-activating protein in resting murine PM which impairs their ability to release adequate amounts of PGE2 and LTB4 after infection with pathogenic E. coli. Even though aging itself does not alter the polarization state of resting PM, they are limited in their capability to adapt functional M1- or M2 phenotypes when subjected to lipopolysaccharide and interferon-gamma or interleukin (IL)-4, respectively. While M1 macrophages become impaired in their abilities to phagocytose bacterial debris and to release cytokines in a temporal manner, we found striking aberrations in the metabololipidome of M2 macrophages with very low levels of SPMs. Our results indicate age-associated alterations of inflammatory pathways in PMs, which persist across ex vivo polarization and impair the temporal release of immunomodulatory mediators during bacterial infection. By combining state-of-the art mass spectrometry-based metabololipidomic and proteomic profiling, we uncovered molecular mechanisms of immunosenescence in TRMs that can be targeted to improve therapeutic strategies for elderly individuals with inflammatory diseases.

ACTIVATION OF SUCNR1 BOOSTS HUMAN MAST CELL REACTIVITY AND ALLERGIC BRONCHOCONSTRICTION

XIAO TANG, Elin Rönnberg, Jesper Säfholm, Madhuranayaki Thulasingam, Mette Trauelsen, Thue W. Schwartz, Craig E. Wheelock, Sven-Erik Dahlén, Gunnar Nilsson and Jesper Z. Haeggström

Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

SUCNR1 is a sensor of extracellular succinate, a Krebs cycle intermediate generated in excess during oxidative stress and has been linked to metabolic regulation and inflammation. While mast cells express SUCNR1, its role in mast cell reactivity and allergic conditions such as asthma remains to be elucidated.

Methods: Cord-blood derived mast cells and human mast cell line LAD-2 challenged by SUCNR1 ligands were analyzed for activation and mediator release. Effects on mast cell-dependent bronchoconstriction was assessed in guinea pig trachea and isolated human small bronchi challenged with antigen and anti-IgE, respectively.

Results: SUCNR1 is abundantly expressed on human mast cells. Challenge with succinate, or the synthetic non-metabolite agonist cis-epoxysuccinate, renders mast cells hypersensitive to IgE- dependent activation, resulting in augmented degranulation and histamine release, de novo biosynthesis of eicosanoids and cytokine secretion. The succinate-potentiated mast cell reactivity was attenuated by SUCNR1 knockdown and selective SUCNR1 antagonists, and could be tuned by pharmacologically targeting protein kinase C and extracellular signal-regulated kinase. Both succinate and cis-epoxysuccinate dose-dependently potentiated antigen-induced contraction in a mast cell- dependent guinea pig airway model, associated with increased generation of cysteinyl-leukotrienes and histamine in trachea. Similarly, cis-epoxysuccinate aggravated IgE-receptor-induced contraction of human bronchi, which was blocked by SUCNR1 antagonism.

Conclusion: SUCNR1 amplifies IgE-receptor induced mast cell activation and allergic bronchoconstriction, suggesting a role for this pathway in aggravation of allergic asthma, thus linking metabolic perturbations to mast cell-dependent inflammation.

COULD MACROPHAGES BE REGULATED BY NEO-DHA?

E. AREL[1,2], C. Oger[2], J.M. Galano[2], T. Durand[2], C. Jopling[3], J.Y. Le Guennec[1], M. Demion*[1]

1) PhyMedExp / 2) IBMM / 3) IGF, Montpellier, France

Context: Macrophages constitute a heterogeneous population of immune cells found in all tissues of the organism which play a key role in the inflammation process. Their activation by lipopolysaccharides (LPS), toll-like receptors ligands, or interferon-gamma (IFNγ), induces the secretion a range of pro-inflammatory mediators (tumor necrosis factor-alpha, cytokines, nitric oxide, reactive oxygen species (ROS)…), driving the inflammation to kill pathogens. This pro-inflammatory profile (M1), switch progressively to an anti-inflammatory profile (M2) to resolve the inflammation process by secreting IL 10 and transforming growth factor beta. Docosahexaenoic acid is an omega-3 fatty acid known to have anti-inflammatory properties by itself and the synthesis of bioactive metabolites such as resolvins, protectins, and maresins. These metabolites resulted of an enzymatic oxidation of DHA and are well studied.

However, in the context of oxidative stress (ROS production) DHA produces non-enzymatic metabolites (named NEO-DHA) such as neuroprostanes and neurofuranes which can have potent anti-inflammatory activities among other things through Peroxisome Proliferator-Activated Receptor (PPAR) activation. We hypothesized that these NEO-DHA are able to regulate macrophages activity by favoring the resolution of inflammation.

Methods: RAW 264.7 cells are deprived of fetal bovine serum one hour before being treated with DHA (10µM), LPS (10ng/mL), IFNγ (100ng/ml) and hydrogen peroxide H2O2 (1mM). Cells are incubated for another hour before viability and ROS production analysis is performed by flow cytometry.

Preliminary data: cell viability test: viability is not affected by treatments: LPS 87%, DHA+LPS 89% or DHA+LPS+IFNγ 83%. However, the addition of H2O2 decreased cell viability from 89 to 80%.
ROS production measurement: ROS production is affected when inflammation is not primed by IFNγ : LPS 24%, DHA+LPS 16%, LPS+IFNγ 23%, DHA+LPS+IFNγ 27%. However, H2O2 addition decreased ROS production from 63 to 58%.

In conclusion, this study would help to determine if DHA and/or NEO-DHA play a role in the process of macrophage activation and/or polarization.

THE IRE1ALPHA INHIBITOR KIRA6 BLOCKS LEUKOTRIENE BIOSYNTHESIS IN HUMAN PHAGOCYTES

Xiao Tang, TARVI TEDER, Bengt Samuelsson and Jesper Z. Haeggström

Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-17177 Stockholm, Sweden

Inflammation and the endoplasmic reticulum (ER) stress take place concurrently in different pathophysiological conditions. Alterations in the ER homeostasis trigger a cascade of ER-mediated intracellular signaling pathways, collectively called the Unfolded Protein Response (UPR), which also results in increased biosynthesis of pro-inflammatory lipid mediators.

In the current study, we investigated the effect of the inhibition of UPR, specifically the Inositol- requiring Enzyme 1 Alpha (IRE1alpha), on the production of leukotrienes. We demonstrated that the potent IRE1alpha inhibitor, KIRA6, blocks the biosynthesis of leukotriene B_4 and cysteinyl leukotrienes in human macrophages and neutrophils which were activated with lipopolysaccharide (LPS) and N-formyl-methionyl-leucyl-phenylalanine (fMLP) or the ER-stress enhancer, thapsigargin (Tg). The production of leukotrienes was attenuated at submicromolar concentration of KIRA6. The production of leukotrienes in IRE1alpha-deficient macrophages was still sensitive to KIRA6 thus indicating that the effect by the inhibitor can be also IRE1alpha-independent. KIRA6 did not have any direct inhibitory effect on key enzymes in the LT pathway, such as phospholipase A2 (PLA2), 5-lipoxygenase (5-LOX), LTA4 hydrolase (LTA4H), and LTC4 synthase (LTC4S), which was determined by the activity measurements with cell lysates. However, KIRA6 dose-dependently blocked the phosphorylation of mitogen-activated protein kinases (MAPKs), p38 and ERK, that play important role in activating cytosolic PLA2 alpha (cPLA2alpha) and 5-LOX. Therefore, the reduction of p38 and ERK phosphorylation resulted in a decreased phosphorylation of cPLA2alpha and attenuated leukotriene production. Furthermore, KIRA6 inhibits p38 activity in vitro which was supported by molecular docking simulations, indicating that the inhibitor can directly interact with the ATP-binding pocket of p38.

In summary, we demonstrated that this potent and unexpected effect of KIRA6 on MAPKs and LT biosynthesis may contribute to the novel immune-modulating properties of this widely used IRE1alpha inhibitor.

A HELMINTH GLUTAMATE DEHYDROGENASE TARGETS EICOSANOID PATHWAYS TO MODULATE TYPE-2 IMMUNITY

SINA BOHNACKER[1], Fiona Henkel[1], Sandra Riemer[1], Franziska Hartung[1], Arie Geerlof[2], André Mourão[2], Jianyang Liu[3], Francesca Alessandrini[1], Antonie Lechner[1], Yannick Schreiber[4], Dominique Thomas[4], Agnieszka M. Kabat[5], Edward Pearce[5], Tarvi Teder[6], Jesper Z. Haeggström[6], David Vöhringer[7], Per-Johan Jakobsson[3], Matthias J Feige[8], Carsten B Schmidt-Weber[1], Julia Esser- von Bieren[1]

1) Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, 80802 Munich, Germany
2) Protein Expression and Purification Facility (PEPF), Institute of Structural Biology, Helmholtz Center Munich, 85764 Neuherberg, Germany
3) Rheumatology Unit, Department of Medicine, Solna, Karolinska University Hospital, 171 77 Stockholm, Sweden
4) Institute of Clinical Pharmacology, Goethe-University Frankfurt, 60590 Frankfurt am Main, Germany
5) Max Planck Institute for Immunobiology and Epigenetics, 79108 Freiburg, Germany
6) Department of Medical Biochemistry and Biophysics, Division of Chemistry II, Karolinska Institutet, 171 65 Stockholm, Sweden
7) Infektionsbiologische Abteilung, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität, 91054 Erlangen-Nürnberg, Germany
8) Department of Chemistry, Technical University of Munich, 85748 Garching, Germany

Bioactive metabolites of arachidonic acid (AA) control chronic inflammation, particularly in therapy-resistant airway disease. Helminth products have been suggested as natural immunoregulators for treating inflammatory diseases. Here, we identified an anti-inflammatory glutamate dehydrogenase (GDH) enzyme in the larval extract of the helminth *Heligmosomoides polygyrus bakeri* (Hpb). We particularly assessed whether Hpb GDH regulates type 2 immune responses (i.e. allergy or anti-helminth immunity) by modulating immune cell metabolism.
Effects of Hpb GDH on the metabolism of monocyte derived macrophages (MDM), were quantified by mediator profiling by LC-MS/MS (eicosanoids, TCA metabolites) and Seahorse analysis. Furthermore, Hpb GDH treated MDM were subjected to RNA sequencing to assess effects on gene expression profile. For characterization of immune regulatory effects in vivo, mice were treated with Hpb GDH during house dust mite (HDM)-induced allergic airway inflammation or during different parasite infections.
In macrophages, Hpb GDH induced the production of prostanoids and 2-hydroxyglutarate (2-HG), which contributed to the suppression of pro-inflammatory cysteinyl leukotrienes. Moreover, Hpb GDH treated MDM showed an induction of regulatory and type 2 suppressive genes, which partially depended on histone acetylation via p300 HAT. Treatment of mice with Hpb GDH attenuated allergic airway inflammation in mice, while the treatment during Hpb infection results in a significant increase in worm burdens.

Our findings thus suggest that helminthic GDH regulates type 2 immune responses by modulating the eicosanoid and TCA metabolite output as well as gene expression in macrophages. Thus, anti- inflammatory modulation of the macrophage metabolism by Hpb GDH may be translated into new immunomodulatory strategies for the treatment of inflammatory diseases.

A POWERFUL OXYLIPIDOMICS PLATFORM TO STUDY INFLAMMATION REGULATION IN COVID-19

DENISE BIAGINI[1] Paolo Oliveri[2] Maria Franzini[3] Tommaso Lomonaco[1] Silvia Ghimenti[1] Andrea Bonini[1] Federico Vivaldi[1] Lisa Macera[3] Laurence Balas[4] Thierry Durand[4] Camille Oger[4] Jean-Marie Galano[4] Fabrizio Maggi[5] Alessandro Celi[6] Aldo Paolicchi[3] and Fabio Di Francesco[1]

1) Department of Chemistry and Industrial Chemistry, University of Pisa, Italy
2) Department of Pharmacy (DIFAR) University of Genova, Italy
3) Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Italy
4) Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, University of Montpellier, CNRS, EBNSCM
5) Department of Medicine and Surgery, University of Insubria, Italy
6) Department of Surgery, Medicine, Molecular Biology, and Critical Care, University of Pisa, and Respiratory Medicine Unit, Pisa Hospital, Italy

Over 400 million reported cases and over 6 million deaths have been globally ascribed to the ongoing coronavirus disease (COVID-19) pandemic. Despite the unprecedented effort of the scientific community, there are still many open questions regarding the pathophysiology of the disease, the therapy, and the management of patients. The key role of inflammation in COVID-19 induced many authors to study the cytokine storm, whereas the role of the oxylipin storm is still poorly understood. Oxylipins are bioactive lipids generated from both ω-3 and ω-6 polyunsaturated fatty acids through enzymatic and non-enzymatic oxidation reactions. During inflammation, oxylipins switch from pro-inflammatory effectors to both anti-inflammatory and specialized pro-resolving lipid mediators, which could promote its resolution.
In the present work, a very powerful analytical platform, based on micro-extraction by packed sorbent (MEPS) coupled to liquid chromatography-tandem mass spectrometry (UHPLC-ESI-MS/MS), was proposed for the determination of 60 plasmatic oxylipins in a single run at ppt levels. Our powerful in-house oxylipidomics platform was successfully employed for a comprehensive characterization of the inflammatory cascade in COVID-19. A chemometric approach was employed to compare inflammatory metabolites coming from both cytokine and oxylipin storms between Intensive Care Unit (ICU) and non-ICU patients.

Unlike cytokines, oxylipins provided such a clear separation between ICU and non-ICU patients, when considered in a multivariate way by principal component analysis. More severe COVID-19 patients showed higher levels of pro-inflammatory isoprostanoids and a selective deficiency of anti- inflammatory and pro-resolving lipid mediators originating from their enzymatic conversion, thus leading to a prolonged and unsolvable pro-inflammatory status. The presence nearby the ICU-cluster of subjects who would have ended up in ICU from 1 to 4 days after the oxylipin quantitation, suggested a potential predictive role of our panel of lipid mediators. A multivariate ROC curve was obtained by application of the UNEQ class-modelling strategy, thus showing an area under the curve equal to 0.92. As far as we know, our observational study is one of the first to suggest the importance of targeting the lipid mediator class switching for a timely picture of the patient ability to respond to SARS-CoV-2 infection

MULTI-STEP SYNTHESES OF A NEW SERIES OF GUT MICROBIAL OCTADECANOID METABOLITES AND EVALUATION OF THEIR PHAGOCYTOTIC ACTIVITY

JOHANNA REVOL-CAVALIER[1,3], Hildur Arnardottir[2], Mats Hamberg[3], Craig E. Wheelock[1]

1) Unit of Integrative Metabolomics, Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden
2) Cardiovascular Medicine Unit, Department of Medicine, Karolinska Institute, Stockholm, Sweden
3) Larodan Research Laboratory, Karolinska Institutet, 171 65, Stockholm, Sweden

Oxylipins are oxygenated compounds that are formed from mono- and polyunsaturated- fatty acids (PUFAs). The most studied class of oxylipins are eicosanoids, which are derived from C-20- containing PUFAs. Given the high abundance of C-18-containing PUFAs in the human diet, C-18- derived oxylipins (termed octadecanoids) are produced at higher concentrations relative to eicosanoids. However, their role in human pathophysiology has only recently started to be investigated and studies suffer from a lack of available analytical standards. There is accordingly a need to synthesize new octadecanoids to facilitate the investigation of their role in health and disease.

Microbes and bacteria are human symbionts that influence the health of their hosts by transforming dietary fatty acids into bioactive molecules. An initial series of 8 known linoleic acid (LA) and alpha- linolenic acid (ALA)-derived microbial metabolites were screened for phagocytotic activity. Two ALA- derived metabolites, 10-hydroxy-12(Z),15(Z)-octadecadienoic acid (10-HODE-ALA) and 10-oxo-12(Z),15(Z)-octadecadienoic acid (10-KODE-ALA), increased phagocytosis activity 30-40% at a concentration of 10-9 and 10-11 M, respectively.

To perform a broader structure activity relationship study, a new series of microbial metabolites was synthesized derived from the C-18 fatty acids oleic acid (OA), LA, ALA, and gamma-linolenic acid (GLA). The series possesses a hydroxyl or a ketone on the 13-position: 13-hydroxy-octadecanoic acid (13-HODA), 13-oxo-octadecanoic acid (13-KODA), 13-hydroxy-9(Z)-octadecenoic acid (13-HOME), 13- oxo-9(Z)-octadecenoic acid (13-KOME), 13-hydroxy-9(Z),15(Z)-octadecadienoic acid (13-HODE-ALA), 13-oxo-9(Z),15(Z)-octadecadienoic acid (13-KODE-ALA), 13-hydroxy-6(Z),9(Z)-octadecadienoic acid (13-HODE-GLA) and 13-oxo-6(Z),9(Z)-octadecadienoic acid (13-KODE-GLA). Metabolites were obtained in 9-to-13 steps, with global yields from 0.7-2.2%, and as racemic mixtures for the hydroxylated metabolites. The developed synthetic strategies will be presented, which used a coupling reaction between halogenated intermediates and terminal alkynes as the key step, followed by a Lindlar hydrogenation to build the unsaturated carbon chains possessing cis-alkene moieties.

The chemotaxis and phagocytosis activity of these new compounds is currently being assayed, and results will be presented. Due to the general unavailability of octadecanoid analytical standards, these compounds will be useful in the development of analytical methods for quantification of these novel microbial metabolites. In particular, these metabolites will be helpful for investigating the role of gut microbiota in transforming dietary fatty acids into bioactive lipid mediators.

MYCOBACTERIUM TUBERCULOSIS-INDUCED PROSTAGLANDIN J2 INHIBITS INFLAMMATORY SIGNALS THROUGH TAK1-NFKB-MAPK PATHWAY IN M1 MACROPHAGES

YUPING NING[1,2], Wenfei Wang[1,2], Stefanie A. Barth[3], Christian Menge[3], Xinchun Chen[2], Oliver Werz[1]

1) Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University, Philosophenweg 14, D-07743 Jena, Germany.
2) Guangdong Provincial Key Laboratory of Regional Immunity and Diseases, Department of Pathogen Biology, Shenzhen University School of Medicine, Shenzhen 518000, China.
3) Friedrich-Loeffler-Institut/Federal Research Institute for Animal Health, Institute of Molecular Pathogenesis, Naumburger Str. 96a, 07743 Jena, Germany

Tuberculosis caused by the pathogen *Mycobacterium tuberculosis* (M.tb) is a leading cause of death globally, where the development of multidrug-resistant mycobacteria further fosters the health concern. In humans, M.tb infects and resides primarily in macrophages. M.tb infection of human macrophages elevates cyclooxygenase-2 (COX-2) expression and upregulates formation of both pro-inflammatory and anti-inflammatory prostaglandins (PGs). The prostaglandins J2 are cyclopentenone containing derivates of PGD_2, which have anti-inflammatory activities. However, whether or not PGJ2 regulates host defense against M.tb infection still remains elusive. In this study, we show that M.tb (H37Rv strain)-conditioned medium (MTB-CM) can stimulate human monocyte- derived M1 macrophages to elevate expression of COX-2 along with enhanced release of PGJ_2. PGJ_2 indeed exerts anti-inflammatory effects by downregulating MTB-CM-induced COX-2 expression and PG formation. We further found that in MTB-CM-stimulated M1 macrophages PGJ_2 decreases the release of pro-inflammatory cytokines, such as IL-6 and IL-1β, while it increases the anti-inflammatory cytokine IL-10 and the M2 marker genes Arg1 and CD163.

Our results show that PGJ_2 mediates these anti-inflammatory effects in M1 macrophages by impairing the activation of the TAK1/NFκB p65/ MAPK pathway, but independent of PPARγ. Together, our findings reveal that M.tb induces PGJ_2 formation in human M1 macrophages with consequently anti-inflammatory effects, which might be exploited for the development of a host-directed therapy (HDT) strategy against tuberculosis.

LEVELS OF EICOSANOIDS IN NASAL SECRETIONS AND URINE ASSOCIATED WITH NASAL POLYP SEVERITY IN CHRONIC RHINOSINUSITIS

AXEL NORDSTRÖM[1], Mattias Jangard[2], Marie Svedberg[1], Michael Ryott[2], Maria Kumlin[1]

1) Department of Health Promotion Science, Sophiahemmet University, Valhallavägen 91, Box 5605, SE-114 86 Stockholm, Sweden.
2) Department of Otorhinolaryngology, Sophiahemmet Hospital, Valhallavägen 91, Box 5605, SE-114, 86 Stockholm, Sweden

Introduction: The nasal mucosal inflammation in patients with Chronic Rhinosinusitis (CRS) is heterogenous. Current literature implicates that arachidonic acid-derived leukotrienes (LTs) and prostaglandins (PGs) may be involved in the pathogenesis. Management of CRS with nasal polyps (NPs; CRSwNP) commonly requires repeated sinus surgery, due to recurrence of NPs. Prior studies rarely consider the extent of NP severity when evaluating the nasal inflammatory milleu and the disease progression in CRS. We sought to assess mucosal and urinary levels of lipid mediators in patients with CRS with different NP severity compared to Aspirin Exacerbated Respiratory Disease (AERD). Lipid mediator levels were also analysed post-surgery over time and compared between NP recurrent and non-recurrent cases.

Methods: Nasal secretions non-invasively collected with swabs in the nostrils and urine samples were obtained pre-surgery from CRS patients without NP (CRSsNP), CRSwNP with or without AERD and non-CRS controls. CRSwNP was further subdivided based on NP score (a measure of NP severity from 0-8); NP-low (≤4) or NP-high (≥5). Levels in nasal secretion of LTE4, LTB4, PGE2, PGD2,15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE) and urinary LTE4 and the PGD2 metabolite 11-beta-PGF2- alpha were quantified by specific immunoassays. Mediator levels were compared between subgroups and correlated to clinical parameters as fractional exhaled nitric oxide (FeNO), blood eosinophil count and smell test score. A subset of patients was followed six- and 12-months post-surgery.

Results: Nasal LTE4 in CRSwNP-AERD were higher as compared to NP-low ($p = .005$). NP-high had elevated levels of nasal PGD2 as opposed to NP-low ($p = .026$). Urinary 11 beta-PGF2 alpha was elevated in NP-high and CRSwNP-AERD as opposed to CRSsNP ($p = .048$, resp. $p = .033$) and correlated with smell test score ($r = -.41$; $p = .045$). Nasal LTE4 correlated to lowered smell test score ($r = -.52$; $p = .001$) as well as to elevated FeNO ($r = .63$; $p = .002$). Results from analyses of mediators post- surgery will also be discussed.

Conclusion: Patients with different NP severity and /or AERD may be distinguished by levels of lipid mediators in nasal mucosa in readily available and non-invasively collected nasal secretions.
Furthermore, lipid mediator levels may relate to the degree of smell loss and FeNO.

ALPHA-HEMOLYSIN FROM STAPHYLOCOCCUS AUREUS IS A POTENT INDUCER OF SPECIALIZED PRO- RESOLVING MEDIATOR GENERATION PROMOTING INFLAMMATION RESOLUTION

PAUL MIKE JORDAN[1], Jana Gerstmeier[1], Simona Pace[1], Antonietta Rossi[2], Cristina González- Estevez[3], Lorena Tuchscherr[4], Charles Nicholas Serhan[5], Oliver Werz[1]

1) Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, 07743 Jena, Germany
2) Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy
3) Cristina Gonzalez-Estevez (Leibniz Institute on Aging, Beutenbergstrasse 11, 07745 Jena, Germany
4) Institute of Medical Microbiology, University Hospital Münster, Domagkstr. 10, 48149 Münster, Germany
5) Charles Nicholas Serhan (Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

Acute inflammation caused by bacterial infections is an essential biological defense mechanism of the host, leading to neutralizing and clearing of invaders and returning to homeostasis. But how the host resolves infectious inflammation, and the underlying mechanisms are incompletely understood. The biosynthesis of specialized pro-resolving mediators (SPMs) is one hallmark of inflammation resolution. SPMs are crucial in promoting resolution of inflammation by enhancing bacterial clearance, tissue repair, and efferocytosis by acting via specific G-protein coupled receptors. *Staphylococcus (S.) aureus* is able to induce SPM formation in mammals. But which virulence factors are involved in the induction of SPM formation is still elusive. We found that intact *S. aureus* but also *S. aureus*- conditioned medium (SACM) induce SPM formation in human M2-like macrophages with the same strength, implicating that liberated virulence factors may induce SPM formation after exposure of the host to pathogenic bacteria. Thereby, we revealed that alpha-hemolysin (Hla), a major exotoxin from *S. aureus*, acts as a potent elicitor of SPM biosynthesis in human M2-like macrophages and also in mouse peritoneum via selective activation of host 15-lipoxygenase-1 (15-LOX-1) by signaling through ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10), the main receptor for Hla. Moreover, we provide evidence that SPM formation in M2-like macrophages is strongly dependent on 15-LOX-1 using selective knock-down of ALOX15A. We further discovered that Hla- deficient *S. aureus* mutants failed to induce SPM formation in M2-like macrophages, implicating a unique effect of Hla in *S. aureus* infections. Furthermore, Hla but not zymosan triggered massive SPM formation devoid of leukocyte infiltration and pro-inflammatory cytokine secretion in the peritoneum of mice in vivo. The importance of SPM in tissue regeneration is demonstrated by lipid mediators derived from Hla-treated M2-like macrophages which in the planarian tissue self-repair model accelerated tissue regeneration. Conclusively, our results unravel novel roles of exotoxins in bacterial infections; thus, besides harming the host, Hla may also exert beneficial functions by stimulating SPM production to promote the resolution of infectious inflammation.

POSTER PRESENTATIONS

POSTER 1

4(RS)-4-F4T-NEUROPROSTANE, PROMISING OXYLIPIN FOR HIGHLY AGGRESSIVE PROSTATE TUMORS TARGETING

ARIANA ABAWI[1], Emma Petiot[1], Giovanna Lollo[2], Thierry Granjon[1], Agnès Girard-Egrot1, Camille Oger[3], Valérie Bultel-Poncé[3], Alexandre Guy[3], Jean-Marie Galano[3], Thierry Durand[3], Ofelia Maniti[1]

1) Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, ICBMS UMR 5246, Univ Lyon, Université Lyon 1, CNRS, F-69622 Lyon, France.

2) Laboratoire d'Automatique, de Génie des Procédés et de Génie Pharmaceutique, LAGEPP UMR 5007, Univ Lyon, Université Lyon 1, CNRS, F-69622 Lyon, France

3) Pôle Chimie Balard Recherche, Institut des Biomolécules Max Mousseron, IBMM, UMR 5247, Université de Montpellier, CNRS, ENSCM. Montpellier, France

Docosahexaenoic acid (DHA, C22:6 n-3) is a fatty acid highly abundant among acyl chains of membrane phospholipids. Upon release from phospholipids, DHA undergoes enzymatic reactions resulting in synthesis of bioactive docosanoids and prostanoids, but also non-enzymatic reactions leading to a more complex pattern of metabolites (isoprostanoids), all of them termed oxylipins. In this study, 12 isoprostanoids which include F1-phytoprostanes, F2-isoprostanes, F3- neuroprostanesDPAn-3 and F4-neuroprostanesDHA derived from ALA (C18:3 n-3), AA (C20:4 n-6), DPA (C22:5 n-3) and DHA respectively were evaluated for their cytotoxic activities using PC-3 cell line from a bone metastasis of grade IV prostatic adenocarcinoma and a control cell line fibroblast. They all show an effect on the PC-3 viability compared to the control cells. Among the tested compounds, the non-enzymatic oxidized metabolite of DHA (NEO-DHA), 4(RS)-4-F4t-neuroprostane (4-F4t-NeuroP), had the most important effect on PC-3 cell viability. To increase the molecule selectivity, we encapsulated 4-F4t-NeuroP in liposomes. By modulating the liposome composition, we designed a set of particles characterized by different membrane fluidities as a key parameter to obtain selective uptake from fibroblast or prostate tumor cells.

In summary, these findings demonstrated that 4-F4t-NeuroP has an anti-tumor activity, affecting the viability of highly aggressive PC-3 cell line, and this activity can be increased by encapsulation in liposomes. Lipid nanoparticles which encapsulate 4-F4t-NeuroP, are an interesting example of drug carriers, as they can be easily designed to promote the fusion of liposomes with their target membrane and ensure drug selectivity.

Keywords: 4(RS)-4-F4t-neuroprostane, PC-3, liposomes, encapsulation

POSTER 2

EFFECTS OF OXYSTEROLS IN IN VITRO MODELS OF INTESTINAL INFLAMMATION

HAFSA AMERAOUI, Juan Bestard-Escalas, Mireille Alhouayek, Giulio G. Muccioli

Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain Av. E. Mounier, 72 - B1.72.01, 1200 Bruxelles - Belgium

Inflammatory bowel diseases (IBD) are a group of life-threatening disorders characterized by a chronic inflammation in the digestive tract. While the available treatments alleviate some symptoms and improve morbidity, patients suffering from IBD can still experience debilitating symptoms affecting their quality of life.

The etiology of IBD is not fully elucidated. It is presumed that they result from an aberrant immune response to the microbiota creating a sustained inflammatory state in genetically predisposed individuals. Like many other autoimmune diseases, gender related differences have been reported. These include for example the prevalence of IBD, the age of onset, as well as response to therapy. Research from the last two decades brought into the spotlight a class of bona fide lipid mediators called oxysterols. Notably these works provided evidence for the immunoregulatory roles of these lipids. We and others previously showed that the levels of several oxysterols (e.g. 25-hydroxycholesterol and 7alpha,25-dihydroxycholesterol) are altered in murine models of colitis as well as in colon biopsies from patients with IBD. Our group also showed that the administration of 4beta-hydroxycholesterol exacerbates DSS-induced colitis while 25-hydroxycholesterol has no effect on the disease's hallmarks.

Here, using different models of intestinal inflammation, we delved into studying the contribution of four oxysterols: 25-hydroxycholesterol, 7alpha,25-dihydroxycholesterol, 25-hydroxycholesterol-3-sulfate and 4beta-hydroxycholesterol to IBD pathogenesis. Using colon explants obtained from DSS-induced colitis mice, we confirmed the pro-inflammatory effects of 4beta-hydroxycholesterol. Interestingly, we observed that 7alpha,25-dihydroxycholesterol and 25-hydroxycholesterol-3-sulfate decreased cytokine production in colon explants from female mice with DSS colitis but not in those from male mice. 7alpha,25-dihydroxycholesterol also decreased cytokine expression in mouse colon organoids, a model of intestinal epithelial tissue. Finally, as macrophages play an important role in gut immune system, we assessed the effect of these four oxysterols on a macrophage cell line (PMA-differentiated THP-1 cells). 7alpha,25-dihydroxycholesterol and 25-hydroxycholesterol-3-sulfate also decreased the activation of THP-1 macrophages induced by lipopolysaccharides and interferon gamma. Our data suggest that 7alpha,25-dihydroxycholesterol could initiate a signaling pathway that results in the dampening of the inflammatory response in macrophages.

Our ongoing works aim to investigate whether this pathway involves GPR183 activation.

POSTER 3

THE RESOLVIN D1 RECEPTOR GPR32 MODULATES HIGH-FAT DIET INDUCED ADIPOSITY MEASURED BY MAGNETIC RESONANCE IMAGING IN HYPERLIPIDEMIC MICE.

HILDUR ARNARDOTTIR[1], Silke Thul[1], Peter Damberg[2], Gonzalo Artiach[1], Magnus Bäck[1,3]

1) Division of Cardiovascular Medicine, Center for Molecular Medicine, Department of Medicine Solna, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden.

2) Karolinska Experimental Research and Imaging Center, Karolinska University Hospital, Stockholm, Sweden.

3) Theme Heart and Vessels, Division of Valvular and Coronary Disease, Karolinska University Hospital, Stockholm, Sweden.

Unresolved chronic inflammation is a key driver of atherosclerosis and related cardiometabolic pathologies. The human GPR32 (hGPR32) receptor is activated by several pro- resolving ligands, which it shares with the receptor ALX/FPR2, to stimulate resolution of inflammation. However, due to the lack of murine homologs of the hGPR32, little is known about its role in vivo. By generating a new hyperlipidemic transgenic mouse model expressing hGPR32 on a Fpr2×ApoE double-KO background (hGPR32myc×Fpr2–/–×Apoe–/–), we showed that hGPR32 is atheroprotective and limits aortic inflammation in vivo (Arnardottir & Thul et al. 2021. JCI). Furthermore, the transgenic mice had significantly lower body weight (BW) compared to the non-transgenic Fpr2–/–×Apoe–/– littermates without any alterations in metabolic features. The aim of this study was to investigate the role of GPR32 in adipose tissue (AT) and its impact on BW in mice. Using our transgenic mouse model and the

STARNET (Stockholm-Tartu Atherosclerosis Reverse Networks Engineering Task) database, we investigated the role of GPR32 in AT associated with cardiometabolic conditions. Female hGPR32myc×Fpr2–/–×Apoe–/– mice had ~20-30% lower BW compared to Fpr2–/–×Apoe–/– littermates that was maintained throughout their lifespan (<12 months). Body fat distribution assessed by magnetic resonance imaging (MRI) showed no difference in total fat (0.91±0.27 vs.0.70±0.05cm3, p=0.977) or VAT (0.50±0.21 vs. 0.34±0.05 cm3, p=0.982) volumes, or the %fat of BW (4.13±0.99 vs. 3.4±0.12 cm3, p=0.911) between the genotypes in chow fed mice. Similar results were observed for the male littermates. High fat diet increased AT volume in both genotypes, however the total fat (14.2±1.01 vs. 9.48±1.0 cm3, p=0.035) and VAT (8.96±1.1 vs. 5.69±0.71 cm3, p=0.0163) aswell as the %fat of BW (39.0±0.65% vs. 32.67±2.3%, p=0.017) were significantly lower in hGPR32myc×Fpr2–/–×Apoe–/–mice compared to Fpr2–/–×Apoe–/– littermates. GPR32 signaling in AT was further supported when interrogating the STARNET containing data on GPR32 expression in both SAT and VAT from patients with coronary artery disease. Taken together, these results indicate that GPR32 does not impact AT distribution under basal conditions, but may have a role in limiting high-fat induced obesity by attenuating AT deposits.

POSTER 4

MODULATION OF CYCLOOXYGENASE-DERIVED PRODUCT FORMATION BY INHIBITION OF GSK 3 BETA AND CDKS IN HUMAN MONOCYTES

VIVIEN BACHMANN[1], Anna Czapka[1], Patrick Schädel[1], Ivan Vilotijevic[2], Oliver Werz[1]

1) Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, 07743 Jena, Germany

2) Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich Schiller University Jena, 07743 Jena, Germany

Prostaglandins (PG) produced by cyclooxygenase (COX)-2 are potent pro-inflammatory lipid mediators (LM) that play crucial roles in inflammation. Currently, non-steroidal anti-inflammatory drugs (NSAIDs) are the major available pharmaceutical therapeutics targeting the biosynthesis of PGE2. Since detrimental side-effects on the gastrointestinal tract and cardiovascular system are common for NSAIDs, the discovery of new potent compounds with different modes of action becomes a necessity. In this context, protein kinases like glycogen synthase (GSK)-3 beta and cyclin-dependent kinases (CDKs) could be suitable targets for the treatment of inflammatory disorders, as they regulate a large number of associated signaling networks. Therefore, research on novel protein kinase inhibitors, such as indirubins, has gained increasing interest in recent years, yet their potential to influence inflammatory processes remains largely unknown. During a structure guided synthesis approach, we introduced substituents mainly in the 5' and 6'-position of indirubins, rendering them suitable for selective inhibition of CDKs and GSK 3 beta, respectively. Here we show the influence of these novel indirubin derivatives and other selective GSK-3 beta inhibitors on PG formation and cytokine release from human monocytes after bacterial challenge. In a structure-activity-relationship analysis, we found that especially 6-bromo-substituted compounds are able to effectively downregulate the formation of pro-inflammatory PGs by inhibiting the expression of cyclooxygenase (COX)-2 in a concentration dependent-manner without marked modulation of other LMs. Furthermore, the compounds decreased the release of major pro inflammatory cytokines like interleukin (IL)-1 beta and tumor necrosis factor (TNF)-alpha. Interestingly, we found a superior inhibition of COX-derived LMs by 5-bromo-substituted indirubin derivatives, which unfortunately correlated with an increase in cytotoxicity. Our results demonstrate that indirubin-derivates are potent protein kinase inhibitors and possess an intriguing therapeutic potential to inhibit pro-inflammatory LM formation via COX-2. Taken together, we described novel protein kinase inhibitors as promising candidates for the therapy of inflammatory diseases and established the close relation between optimization of compound structures and their activities in the cellular context.

POSTER 5

INTERACTIONS BETWEEN PROSTACYCLIN AND NITRIC OXIDE PATHWAYS MODULATORS IN HUMAN PULMONARY ARTERIES DERIVED FROM PATIENTS WITH OR WITHOUT PH

THOMAS BECK[1], Achraf Boulfrad[2], Asmaa A. Khalifa[3], A. Hichem Badji[2], Heba Abdelazeem[2,4], Adam Silverstein[5], Dan Longrois[2.6] and Xavier Norel[2]

1) Sorbonne Université, INSERM, LVTS, F-75018 Paris, France
2) Université Paris Cité and Université Sorbonne Paris Nord, INSERM, LVTS, F-75018 Paris, France
3) Pharmacology and Therapeutics Department, Faculty of Pharmacy, Pharos University in Alexandria, Egypt
4) Dept. Pharmacology & Toxicology, Faculty of Pharmacy, Alexandria University, Egypt
5) United Therapeutics Corporation, Research Triangle Park, NC 27709, USA
6) Hôpital Bichat-Claude Bernard, AP-HP, Université Paris Cité, Paris, France

Introduction: Pulmonary hypertension (PH) secondary to a lung disease (group-III) is characterized by an elevation in the mean pulmonary artery pressure (>20 mmHg) and high mortality. The classical mechanisms are a reduced synthesis of prostacyclin (PGI_2) and nitric oxide (NO) in human pulmonary artery (HPA). PGI_2 analogues [iloprost (ILO), treprostinil (TRE)], NO pathway activators (Sildenafil (SILD)) and combinations are PH therapeutics; however, there are few studies comparing their effectiveness in isolated HPA.

Aim of the study: to investigate the effect of combination treatments (PGI_2 analogues +/- SILD) on cAMP and cGMP content, on the enzymes responisble for PGI2 and NO synthesis, and on the vasorelaxations of HPA derived from patients +/-PH group-III.

Methods: HPA from non-PH (n=8) and PH group-III patients (n=3), were incubated (18h) with SILD, ILO, TRE, SILD+ILO or SILD+TRE (10^{-6}M - 10^{-5}M). PGI2-synthase (PGIS) and endothelial NO-synthase (eNOS) protein expressions were quantified by Western blot. cAMP, cGMP and/or 6ketoPGF1α (stable metabolite of PGI_2) levels were analysed by ELISA. An organ bath system was also used to measure changes in HPA vasorelaxations induced by these drugs. Molecules are from Cayman-Chemical.

Results: cAMP content in HPA from PH-patients (without stimulation) was significantly lower than HPA from non-PH patients, a similar tendency was observed for cGMP. (SILD+ILO) stimulations decreased the cAMP content in comparison to ILO alone. In contrast, (SILD+TRE) stimulation increased cAMP concentration compared to TREP alone. In non-PH patients HPA, an additive increased cGMP content was obtained with (SILD+ILO) or (SILD+TRE) treatments in comparison to ILO or TRE treatments alone. The enzymes (PGIS and eNOS) expressions were slightly increased depending of the treatments. Vasorelaxations of HPA were reduced with SILD+ILO stimulation in comparison to those induced by ILO alone. SILD+TRE versus TRE vasorelaxations were not significantly different.

Conclusion: Only in HPA from PH patients, cAMP content was modulated by SILD and/or PGI_2 analogue, while changes of cGMP were only detected in non-PH HPA. The effects of combination treatment were strongly different between ILO and TRE when used in combination with SILD. These preliminary results are less in favor of the use of ILO+SILD in terms of HPA vasorelaxation.

POSTER 6

EVALUATION OF ENDOCANNABINOID DERIVATIVES ON THE PRO-INFLAMMATORY ACTIVATION OF COLON EPITHELIAL CELLS

JOAN BESTARD-ESCALAS, Hafsa Ameraoui, Mireille Alhouayek, Giulio G. Muccioli

Av. E. Mounier, 72 - B1.72.01, 1200 Brussels - Belgium

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory disorders of the gastrointestinal tract collectively described as inflammatory bowel diseases (IBD). While rarely lethal, IBD shows increasing prevalence and incidence and profoundly affects patients' quality of life. The therapeutic strategies available to control the symptoms of IBD produce important side effects and progressively lose their efficacy. Thus, there is a need to explore new ways to modulate IBD.

Bioactive lipids stand up as important modulators of inflammation. For instance, endocannabinoids, and their COX-2-derived metabolites, are anti-inflammatory and pro-apoptotic. Besides fatty acids, COX-2 can also metabolize the endocannabinoids 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (AEA), thus producing glycerol ester (PG-Gs) and ethanolamide (PG-EAs) derivatives, respectively, of the prostaglandins. PG-Gs and PG-EAs, although barely studied, are promising lipid mediators to modulate inflammation. We have shown that PGD_2-G decreases macrophage activation and has anti-inflammatory properties in the colon. However, not all the PG-Gs and PG-EAs exert anti-inflammatory effects. For instance, PGE_2-G possesses pro-inflammatory properties. Also, PGD_2-EA induces apoptosis in colorectal cancer (CRC) cell lines.

When epithelial cells are stimulated with LPS and INFγ, NF-kB translocates to their nucleus activating the transcription of pro-inflammatory cytokines, such as IL-8 or MIP2α. To understand better the role of PG-Gs and PG-EAs in the inflammation of colon epithelial cells, we used two colorectal cancer cell lines (HT29 and Caco2) and primary mouse colon organoids. PGD_2, PGD_2-G, and PGD_2-EA enhanced the nuclear translocation of NF-kB in HT29 cells. Also, they increased the expression of IL-8 in HT29 and Caco2 cells, as evaluated by ELISA and qPCR. Similarly, these molecules increased the expression of KC (a mouse homolog for IL-8) in mouse colon organoids, although not significantly.

The enhancement of inflammatory signals in these epithelial models contrasts with their previously described anti-inflammatory role indicating a different effect of PGD_2, PGD_2-G, and PGD_2-EA in epithelial and immune cells.

Thus, our results point out the necessity to delve into the effects of PG-Gs and PG-EAs in colon epithelial cells and their potential role in the interaction between epithelial and immune cells in colon inflammation.

POSTER 7

A MOLECULAR DYNAMICS WORKFLOW FOR INTERROGATING PUFA-DERIVED LIPID MEDIATOR INTERACTIONS WITH THE TRPV1 CHANNEL

NADA BIRKIC, Zeljko Svedruzic, and Christian A. Reynolds

Department of Biotechnology, University of Rijeka, Rijeka, Croatia

Various fatty acyl lipid mediators derived from dietary polyunsaturated fatty acids (PUFAs) modulate nociception. Many of these lipid mediators can directly bind ion channels located on sensory nerves, including the transient receptor potential vanilloid 1 (TRPV1) channel.

Linoleic acid, the most abundant PUFA in the modern diet, is a precursor of many pronociceptive lipid mediators with potential for TRPV1 binding, and excessive dietary linoleic acid intake is associated with exaggerated nociceptive hypersensitivity. Although recommendations about dietary fatty acid intake exist for some diseases (e.g., cardiovascular disease), the role of dietary fatty acids in promoting pain disorders is not completely understood. Molecular dynamic simulation offers a powerful strategy for interrogating lipid mediator-TRPV1 interactions. All-atom simulations of the TRPV1 channel were prepared using CHARMM-GUI Membrane Builder and OPMprotocols were used to position and orient the channel in a membrane bilayer. The composition in the membrane bilayer in this all-atom system is as follows: 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE), 1-palmitoyl-2-oleoyl-phosphatidic acid (POPA), 1-palmitoyl-2-oleoyl-phosphatidylserine (POPS), N-palmitoyl-sphingomyelin (PSM), 1-palmitoyl-2-oleoyl-phosphatidylinositol (POPI), cholesterol (CHOL). Multi-scale molecular dynamics simulations were used to identify amino acid residues that are most affected by lipid mediator binding in each of the four subunits. Each step in the peristaltic motion was described down to the individual atoms using all-atom approaches and statistical analysis of the correlated motion between sub-units. Each molecular dynamic simulation of lipid mediator-bound TRPV1 structures was compared to the TRPV1 structure with no bound agonist and to the capsaicin-bound TRPV1 structure. Taken together, this molecular dynamic workflow can help elucidate molecular mechanism underlying modulation of nociception by fatty acyl lipid mediators.

POSTER 8

EFFECTS OF N-ACYLETHANOLAMINE-HYDROLYZING ACID AMIDASE INHIBITION ON LUNG INFLAMMATION

PAULINE BOTTEMANNE[1], Adrien Paquot[1], Alexandros Makriyannis[2], Mireille Alhouayek[1] and Giulio G. Muccioli[1]

1) Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, UCL, B-1200 Brussels, Belgium
2) Center for Drug Discovery, Northeastern University, Boston, MA 02115, USA

Abstract: N-acylethanolamines (NAEs) are endogenous bioactive lipids linked to the endocannabinoid system. They are implicated in numerous biological processes including pain and inflammation. Some NAEs, such as the anti-inflammatory compound N-palmitoylethanolamine (PEA) and the endocannabinoid N-arachidonoylethanolamine (AEA), have been more studied than others; however other single NAE species can also reduce LPS-induced macrophage activation.
The levels of NAEs and therefore their actions can be controlled by modulating the activity of their two hydrolytic enzymes, fatty acid amide hydrolase (FAAH) and N-acylethanolamine acid amidase (NAAA). Given that much more is known about FAAH, we decided to investigate how lung inflammation affects NAAA expression and NAE levels. We then wanted to investigate if NAAA inhibition could increase NAE levels in vivo and thus decrease macrophage activation. The effects of NAAA inhibition were studied in primary alveolar macrophages and neutrophils to see if NAAA inhibition could be an interesting strategy to decrease lung inflammation.

Effects of lung inflammation on NAE levels and NAAA expression were studied in three different murine models. Inflammation was induced either by lipopolysaccharides (i.p. injection during 10days or a single i.t. administration) or house dust mites intra nasal administrations. The latest model was used to mimic allergic inflammation. Alveolar macrophages and PBMC were activated by incubation with for 8hours. The NAAA inhibitor (AM9053) or vehicle were added one hour prior to LPS. Neutrophils were co-incubated with LPS and AM9053 for 4hours. mRNA expression of pro-inflammatory cytokines and enzymes was measured by RT-qPCR. Levels of pro-inflammatory cytokines were measured by ELISA in the medium of cells. NAEs were quantified by HPLC-MS.

Our data show that lung inflammation increased NAAA expression while having few effects on NAE levels. Moreover, administration of AM9053 to mice with chronic LPS-induced inflammation exerts anti-inflammatory effects in the lung. NAAA inhibition also decreases LPS-induced activation of murine and human alveolar macrophages as well as LPS-activated PBMC and murine neutrophils.

This work highlights the beneficial effects of NAEs and NAAA inhibition on macrophage activation and shows a protective effect of NAAA inhibition in vivo on lung inflammation. Therefore, these data support NAAA inhibition as an interesting anti-inflammatory strategy.

POSTER 9

INCREASED METABOLISM OF MARESIN IN CARDIAC LEFT VENTRICLE OF PATIENTS WITH HEART FAILURE

BOUHADOUN A[1], Manikpurage HD[2], Baillif V[3], Dubourdeau M[3], Longrois D[1,4] and NOREL X[1]

1) Université Paris Cité and Université Sorbonne Paris Nord, INSERM, LVTS, F-75018 Paris, France
2) Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec, Université Laval, Québec, Canada
3) Ambiotis SAS, 31400 Toulouse, France. 4/HÃ´pital Bichat-Claude Bernard, AP-HP, Université de Paris, Paris, France

Cardiac fibrosis is a major cause of heart failure (HF). The balance between inflammation and resolution is crucial in the pathogenesis of remodeling and thus for cardiac functionality. The production, the metabolic pathway and the role of specialized pro-resolving mediators (SPM: resolvin (Rv), protectin (PD) and maresin (MaR) in the myocardial inflammatory response are lacking.

Aims: Using heart left ventricle (LV) samples obtained from donors and HF patients, we have compared the production of some docosahexaenoic acid (DHA) -derived metabolites. We have also investigated the MaR metabolic pathway in LV: the receptor LGR6 and the biosynthetic enzymes lipoxygenase enzymes: 15-LOX-1, 15-LOX-2 and 12-LOX.

Methods: Fresh LV samples derived from n=16 patients with or without HF were incubated (18h in RPMI with antibiotics, 70 mg LV/ml) with or without DHA (0.1 mM) before storage at -80°C. After homogenization of the samples, concentrations of protein were measured by BCA assay and levels of RvD1-5, PDX, PD1, MaR1-2 and 7S-MaR1 (an epimer of the active MaR1) were measured by LC-MS (Ambiotis SAS). Results are expressed in pg/µg of proteins, and statistical analysis were performed using 2 way-ANOVA. In addition, in these samples, we measured mRNA expression level of MaR1 receptor (LGR6) and ALOX15A by RT-qPCR.

Results: Without stimulation, RvD1-4 and Mar2 were the lowest concentrations measured in LV while the highest were 20-30 fold greater: RvD5 0.13±0.04 pg/µg and MaR1 0.24±0.10 pg/µg. After stimulation with DHA the productions of RvD, MaR and PD were significantly increased. This increase in LV preparations derived from heart failure patients were significantly 3 times greater (versus non-heart-failure patients derived samples) only for MaR1 and 7S-MaR1. The RT-qPCR experiments showed that gene mRNA expression of ALOX15 and LGR6 in LV samples derived from HF patients are significantly increased (n=7-8). Our immunofluorescence results (n=4) showed that 15-LOX-1, 15-LOX-2 and 12-LOX are expressed in cardiomyocytes.

Conclusions: Our results suggest that the increased production of MaR1 and 7S-MaR1 and the MaR1-receptor expression may have an important role during the resolution phase in human heart failure.

POSTER 10

15-LIPOXYGENASES LIPID PRODUCTS MARK DISEASE PROGRESSION IN MULTIPLE SCLEROSIS

J.Y. BROOS, F.C. Loonstra, R. Ruiter, W.H. Fung, M. Heijink, J. Killestein, B.M.J. Uitdehaag, M.A. Giera, H.E. De Vries, G. Kooij

Boelelaan 1117, 1081 HV Amsterdam

Disturbances in lipid mediators belonging to the omega-6 lipid pathway have been observed in Multiple Sclerosis (MS), where they are believed to be related to an uncontrolled or chronic neuro- inflammatory response. However, as lipid mediators are also involved in various other physiological processes, we hypothesized that disturbances in this and/or other lipid pathways could also relate to different pathological processes as observed in MS patients. To address this hypothesis, we here investigated bioactive lipids belonging to the ω-3 / ω-6 lipid classes in MS by using a lipidomics approach on a unique patient cohort (Project Y) consisting of primary progressive MS (PPMS), relapse-remitting MS (RRMS) or secondary progressive MS (SPMS) patients all born in the same year: 1966. This is essential as age normally is one of the biggest confounding factor in MS research.

Method: Lipidomic analysis was performed on plasma samples of control subjects (n = 125) and patients with RRMS (n = 169), PPMS (n = 36) and SPMS (n = 80) using a HPLC MS/MS. Besides plasma collection, clinical parameters including Expanded Disability Status Scale (EDSS), neurofilament light (NFL; biomarker for axonal damage), glial fibrillary acidic protein (GFAP; astrocyte activation marker) concentration and amount/location of brain lesions and atrophy (MRI) were obtained from this unique patient cohort.

Results: Several lipid mediators were found to be upregulated, predominantly, in the SPMS group compared to healthy controls, most of which showed an association with enzymes belonging to the lipoxygenases (LOX) enzyme family. Correlations between these plasma lipid levels and clinical parameters such EDSS, sNFL and GFAP revealed a potential involvement of these lipids in neurodegeneration and astrocyte activation. In addition, the ALOX-15B associated lipid 15(S)-HETE positively correlated with atrophy in several brain areas of SPMS patients and lesion volume, highlighting its potential involvement in progressive MS pathology.

Conclusion: Changes in lipids belonging to the omega-6 lipid pathway are predominantly observed in progressive MS patients and could relate to key processes in MS pathogenesis including neurodegeneration.

POSTER 11

LIPID MEDIATOR PROFILING REVEALS DISTINCTIVE EICOSANOID SIGNATURES IN LEUKOCYTES AND PLATELETS TREATED WITH GINSENOSIDES

VERA BRUGGINK[1], R.K. Hofstetter[1], O. Werz[1], D. Kralisch[2]

1) Institute of Pharmacy, Philosophenweg 14,07743 Jena, Germany
2) JeNaCell, Göschwitzer Str. 22, 07745 Jena, Germany

Eicosanoids are essential lipid mediators involved in modulating the immunological response and cardiovascular system, which - upon dysregulation - are implicated in inflammatory and cardiovascular disease. Utilizing a limited range of polyunsaturated fatty acids (PUFA), blood cells generate a wide spectrum of distinct eicosanoid species, resulting in the formation of characteristic lipid mediator profiles.

While these profiles are cell-type and stimulus-dependent, they are also amenable to pharmacological intervention, including natural products. Ginsenosides, glycosides isolated from the traditional medicinal plant Panax ginseng, are known to exert a wide variety of biological effects, including the downregulation of pro-inflammatory prostaglandins, but little is known about their effect on the eicosanoid profile at large or on specialized pro-resolving mediators (SPM). Here, we use UHPLC-MS/MS-based metabololipidomics with human polymorphonuclear leukocytes (PMNL), macrophage phenotypes (M0/M1/M2), and platelets treated with ginsenosides to reveal distinctive lipid mediator signatures depending on the cell-type, treatment period, and stimulus.

Furthermore, we evaluated the expression of various lipid mediator-biosynthetic enzymes, such as cyclooxygenases (COX) and lipoxygenases (LOX), on both mRNA and protein level, as well as the activity of these enzymes in response to ginsenosides. Interestingly, marked differences were demonstrated between various ginsenosides, including compound K, which resulted in the general downregulation of COX and LOX products; ginsenoside Rg3, which affected PUFA liberation and the upregulation of LOX products (including SPM); and the existence of inert ginsenosides such as Rg1.

These results demonstrate the utility of metabololipidomics by helping to explain how Panax ginseng can exert a vast range of biological activities - and indeed opposite effects - depending on the type of extract used, the treatment duration, and diagnosis. Ultimately, this delineation between active and inactive ginsenosides may aid in the development and targeted use of natural products for the treatment of inflammatory and circulatory diseases.

POSTER 12

NOVEL ENDOGENOUS VITAMIN E METABOLITES WITH STRONG ACTIVITY AGAINST 5-LO AND MPGES-1 - A DUAL INHIBITOR STUDY

ELENA BRUNNER[1], Markus Werner[1], Stephan Permann[3], Laura Fritsch[1], Paul Jordan[1], Khaled Alsabil[2], Guillaume Viault[2], Denis Séraphin[2], Pascal Richomme[2], Jean-Jacques Helesbeux[2], Andreas Koeberle[1], Oliver Werz[1],[3]

1) Philosophenweg 14, 07743 Jena, Germany
2) CMBI, University of Innsbruck, 6020 Innsbruck, Austria
3) Univ Angers, SONAS, SFR QUASAV, F49000 Angers, France

Inflammation is a cause for many chronic and non-chronic widespread diseases including asthma, rheumatoid arthritis and atherosclerosis. This is partially caused by an imbalance in the arachidonic acid cascade towards leukotrienes, produced by 5-lipoxygenase and/or prostaglandins (PG) synthesized via the cyclooxygenase (COX) pathway. Nowadays, non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit cyclooxygenase are the primary therapeutics, even though they are associated with severe adverse side effects especially in long term use due to the inhibition of homeostatic PGs. Gastrointestinal complications and asthma caused by NSAIDs could be avoided by using a smart targeting approach, that is, aiming at inhibiting both the COX-downstream enzyme microsomal prostaglandin E2 synthase-1 (mPGES-1) and 5-LO. Since inhibition of one single enzyme would cause a shunt to the other pathway, dual inhibition of 5-LO and mPGES-1 may be a more beneficial approach. Endogenous vitamin E metabolites in this sense have proven themselves as potent 5-LO inhibitors. By screening a library of semi-synthesized vitamin E derivatives using cell-free and cell-based assays, we aimed at identifying dual inhibitors of 5-LO and mPGES-1. Then, we tested the most potent hits in additional test systems for inhibition of COX1, COX2, and soluble epoxide hydrolase as well as for broad lipid mediator (LM) profiles, cytokines release, macrophage phenotype surface markers, and LM-biosynthetic enzyme expression in different immune cells. Finally, we assessed the bioactivity of these hit compounds in human whole blood to confirm the pharmacological relevance of the proposed target interaction. In parallel further screening studies are ongoing, revealing even more potent compounds. With these findings we hope to get closer to get promising anti-inflammatory drugs that displays dual inhibition of the two crucial enzymes with less adverse side effects.

POSTER 13

ARACHIDONIC ACID METABOLISM IN NASAL POLYPS.

ENRICO BUONAMICO[1,2], Roberta Salerno[1], Domenico Giorgio Macchia[1], Maria Pia Rossi[1], Lucia Anna Giliberti[1], Giovanna Elisiana Carpagnano[2],
Maria Filomena Caiaffa[3], Luigi Macchia[1]

1) School and Chair of Allergology and Clinical Immunology, University of Bari, Bari, Italy
2) School and Chair of Respiratory
Disease, University of Bari, Bari, Italy
3) Chair of Allergology and Clinical Immunology, University of Foggia, Foggia, Italy.

Nasal polyposis is currently the focus of intensive research, since this condition has been recognized as one of the most prominent expression of the type-2 inflammatory endotype, along with severe asthma and atopic dermatitis. Moreover, innovative monoclonal antibody-based therapies, such as dupilumab, have been recently approved by the regulatory Agencies for this disease.
Here, we report on investigations on arachidonate metabolism and expression and activity of relevant enzymes in nasal polyps.

Thus, 19 polyp specimens (from 14 patients), obtained by surgical excision, were studied by RT-PCR, Western blotting and enzyme assay, performed in polyp homogenates.

Results. RT-PCR revealed a discrete expression of 5-LO: 0.284 A.U. ± 0.220, by using beta-actin as the reference constitutive gene. Expression of COX-1, COX-2 and LTC4-syntase was also investigated. Accordingly, a substantial expression of immune-reactive 5-LO was detected by Western blotting. In contrast, only modest amounts of immune-reactive COX-1 and COX-2 were identified. However, by a competitive ELISA assay, we demonstrated that a measurable cycloxigenase activity was present in polyp homogenates, as documented by PGE_2 formation (from 2.9 to 140 pg/mg of protein). The amounts of PGE2 measured by this assay increased up to 20-fold if the samples were incubated in the presence of exogenous arachidonic acid (50 micromolar). The biosynthesis of lypoxigenase derivatives was studied by RP-HPLC, upon incubation of homogenates with exogenous arachidonic acid (50 micromolar). Discrete amounts of 15-HETE and 12-HETE were detected, suggesting that both 15-LO and 12-LO are expressed in this pathologic material. Finally, RP-HPLC also revealed the presence of two peaks of unknown origin.

These data indicate that arachidonic acid derivatives play a not negligible role in the much complex scenario of the type-2 inflammation, which characterizes nasal polyposis.

POSTER 14

STUDY OF THE CONVERSION OF LEUKOTRIENE A4 TO LEUKOTRIENE B4 DRIVEN BY LEUKOTRIENE A4 HYDROLASE

MIQUEL CANYELLES-NIÑO, Àngels González-Lafont, José María Lluch

Departament de Química, C/ dels Til·lers, s/n, Bellaterra (Cerdanyola del Vallès) 08193, Barcelona, Spain

LTA4H is a bifunctional zinc metalloenzyme that converts leukotriene A_4 (LTA_4) into leukotriene B4 (LTB4), one of the most potent chemotactic agents involved in acute and chronic inflammatory diseases. In this reaction, LTA4H acts as an epoxide hydrolase with a unique and fascinating mechanism, which includes the stereoselective attachment of one water molecule to the carbon backbone of LTA_4 several methylene units away from the epoxide moiety.

By combining Molecular Dynamics simulations and Quantum Mechanics/Molecular Mechanics calculations, we obtained a very detailed molecular picture of the different consecutive steps of that mechanism. By means of a rather unusual 1,7-nucleophilic substitution through a clear SN1 mechanism, the epoxide opens and the triene moiety of the substrate twists in such a way that the bond C6-C7 adopts its cis (Z) configuration, thus exposing the R face of C12 to the addition of a water molecule hydrogen-bonded to ASP375. Thus, the two stereochemical features that are required for the bioactivity of LTB_4 appear to be closely related. The noncovalent π-π stacking interactions between the triene moiety and two tyrosines (TYR267 and, especially, TYR378) that wrap the triene system along the whole reaction explain the preference for the cis configuration inside LTA4H.

POSTER 15

ANALYSIS OF POLAR LIPIDS IN PLASMA OF OMEGA-3 PUFA SUPPLEMENTED HUMAN SUBJECTS BY COMBINATION OF UNTARGETED LC-HRMS/MS AND TARGETED LC-MS/MS

LAURA CARPANEDO[1], Katharina M. Rund[1], Tim Wermund[1], Philip C. Calder[2], Nils Helge Schebb[1]

1) Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Germany
2) Faculty of Medicine, University of Southampton, United Kingdom

The composition of lipids is highly dynamic and affected by the diet. Long chain omega-3 polyunsaturated fatty acids (PUFA) present in fish oils such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential nutrients showing anti-inflammatory properties. n3-PUFA supplementation leads to a dose dependent change of the fatty acid (Am. J. Clin. Nutr.,2012, 96, 748) as well as oxylipin pattern in the blood (Am. J. Clin. Nutr.,2019, 109, 1251). However, information about the lipid species which are thereby strongest modulated are scarce.

Here, plasma from human subjects receiving n3-PUFA capsules (EPA and DHA) corresponding to 4 portions of fatty fish per week over 12 months was analyzed. Lipids at baseline and following 12 months were measured by untargeted lipidomics using liquid chromatography (LC)-high resolution mass spectrometry (HRMS) and semi-quantified using SPLASH internal standards (IS). The lipids which were modulated strongest after n3-PUFA supplementation were then quantified by means of targeted LC-MS/MS. Lipids were extracted from plasma by liquid-liquid extraction after addition of IS using methanol and methyl tert-butyl ether.

For both methods, the chromatographic separation was achieved using an Acquity Premier CSH C18 column (2.1 x 100 mm, 1.7 µm). For untargeted lipidomics, samples were analysed using a Q Exactive HF within two separate runs for positive and negative mode. Data were evaluated using MS-DIAL. Targeted analysis was performed on a QTRAP 6500+ MS, after negative electrospray-ionization in multiple reaction monitoring mode using specific ion transitions.

Combination of untargeted lipidomics and targeted LC-MS/MS allowed to characterize the lipid profile modulated in response to n3 PUFA supplementation and to select those which are modulated strongest for accurate quantification. Following n3-PUFA supplementation most pronounced changes were observed in plasma phosphatidylcholines and EPA containing lipids were strongest elevated.

POSTER 16

ALBUMIN LIPIDOMICS REVEALS MEANINGFUL COMPOSITIONAL CHANGES IN ADVANCED CIRRHOSIS AND ITS POTENTIAL TO PROMOTE INFLAMMATION RESOLUTION.

MIREIA CASULLERAS[1,2], Roger Flores-Costa[1,2], Marta Duran-Güell[1,2], Ingrid W. Zhang[1,2], Anna Curto[2], Cristina López-Vicario[1,2], Esther Titos[1,3], Javier Fernández[2,4], Vicente Arroyo[2] and Joan Clària 1,2,3

1) Biochemistry and Molecular Genetics Service, Hospital Clínic-IDIBAPS-CIBERehd, Barcelona, Spain
2) European Foundation for the Study of Chronic Liver Failure (EFClif) and Grifols Chair, Barcelona, Spain
3) Department of Biomedical Sciences, University of Barcelona, Barcelona, Spain; 4-Liver Unit, Hospital Clínic, Barcelona, Spain.

Albumin infusions are therapeutically used to revert hypoalbuminemia and to replace the extensively oxidized albumin molecule circulating in patients with acutely decompensated (AD) cirrhosis. Since albumin has high affinity for lipids, here we characterized the albumin lipidome in AD patients and explored the albumin effects on the release of fatty acid (FA)-derived lipid mediators by peripheral leukocytes.

Lipids and lipid mediators were measured by LC-MS/MS in albumin-enriched and albumin-depleted plasma fractions separated by affinity chromatography and in leukocyte incubations from 18 AD patients and 10 healthy subjects (HS).Lipid mediators were also measured in 41 AD patients included in an albumin therapy trial. The plasma lipidome associated with AD cirrhosis was characterized by generalized suppression of all lipid classes except FAs. In contrast to HS, albumin from AD patients had lower content of polyunsaturated FAs (PUFAs), especially of the omega-3-PUFA docosahexaenoic acid. Consistent with this, the PUFA-derived lipid mediator landscape of albumin from AD patients was dominated by lower content of monohydroxy FA precursors of anti-inflammatory/pro-resolving lipid mediators (i.e. 15-hydroxyeicosatetraenoic (15-HETE)). In addition, albumin from AD patients was depleted in prostaglandin (PG) E_2, suggesting that this proinflammatory PG mainly travels disassociated to albumin in these patients. Incubation of leukocytes with exogenous albumin reduced PG production while inducing 15-lipoxygenase expression and 15-HETE release. Similar effects were seen under lipopolysaccharide plus N-formylmethionyl-leucyl-phenylalanine-stimulated conditions. Finally, PG levels were lower in AD patients receiving albumin therapy whereas 15-HETE was increased after albumin treatment compared to baseline.

Conclusion: Our findings indicate that the albumin lipid composition is severely disorganized in AD cirrhosis and that administration of exogenous albumin has potential to redirect leukocyte biosynthesis from pro-inflammatory to pro-resolving lipid mediators.

POSTER 17

OMEGA-3 SUPPLEMENTATION AND BIOACTIVE LIPID MEDIATORS FOR THE CONTROL OF JOINT INFLAMMATION

CHIARA CECCONELLO, Mauro Perretti, Lucy V. Norling, Jesmond Dalli

The William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, United Kingdom

The aim of this study is to establish the efficacy of omega-3 supplementation in settings of inflammatory arthritis and to investigate the cellular and morphological effects, with the ultimate aim to associated them to specific lipid mediators. Mice were treated with K/BxN arthritic serum injection and fed either with normal, Omega-3-enriched or western diet for two weeks prior to and throughout the duration of experimental arthritis. In the omega-3 -enriched diet group, mice exhibited reduced i) arthritic score, ii) loss of weight when compared to normal or western diet group, respectively. Paw oedema was reduced in omega-3 mice, as compared to both normal and western diet receiving mice. These macroscopic changes were associated with regulation of leukocytes and fibroblasts phenotypes. Specifically, at peak of arthritis, the pro-resolution macrophages MerTK+CD206+ and the protective CX3CR1+ macrophage subsets were increased in the omega-3 enriched diet group, compared to animals on normal or western diet. Lipid mediator profiling of the arthritis paws identified higher levels of EPA-derived RvE2 and RvE4 in omega-3-enriched diet animals. Initial cellular analyses revealed that RvE2 and RvE4, used alone or in combination, promoted anti-catabolic effects in C28/I2 chondrocytes micromasses. In vivo experiments in K/BxN-serum treated mice are ongoing.

Collectively these data identify novel molecular and cellular determinants of the beneficial properties of omega-3 diet supplementation in preclinical settings, opening avenues for innovative therapeutic approaches for the treatment of people with RA.

POSTER 18

SUSTAINABLE EXTRACTS OF MICROALGA CHLOROCOCCUM AMBLYSTOMATIS RICH IN OMEGA-3 LIPIDS WITH ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL FOR SKIN DISEASES

TIAGO CONDE, Daniela Couto, TÃ¢nia Melo, Ana S. P. Moreira, Paula Ferreira, Margarida Costa, Joana Silva, Bruno Neves, Pedro Domingues, Maria R. Domingues

Centre for Environmental and Marine Studies, CESAM, Department of Chemistry, Santiago University Campus, University of Aveiro, 3810-193 Aveiro, Portugal

The prevalence of inflammatory skin diseases continues to increase impairing the daily lives of the affected children and adults. Chronic inflammatory response, overproduction of pro-inflammatory mediators and reactive oxygen species (ROS) are involved in the pathophysiology of these diseases. Algae lipids are well-known natural antioxidant and anti-inflammatory agents, and some have been applied to modulate skin diseases. However, the comprehension of the relationship between algae lipids and their bioactive potential remains scarcely studied. In this work, we evaluated the antioxidant and anti-inflammatory potential of freshwater microalga *Chlorococcum amblystomatis* extracts obtained with a sustainable and green methodology using ethanol and ultrasonication (UAE). The composition in polar lipids were evaluated by liquid-chromatography mass spectrometry (LC-MS) to understand the relation between lipid composition and bioactivity. The UAE extracts of *C. amblystomatis* revealed high antioxidant capacity evaluated through inhibition of the ABTS and DPPH radicals. In addition, these lipid extracts showed anti-inflammatory potential through cyclooxygenase-2 (COX-2) inhibition, both important to treat inflammatory skin diseases, like eczema or atopic dermatitis [1].

The UAE extracts of *C. amblystomatis* had a higher purity of lipids (91.8±4.5 %) in relation to extraction with chlorinated solvent (81.2±4.0). The polar lipid profile revealed a high abundance in diacylglyceryltrimethylhomo-serine (DGTS) and monogalactosyldiacylglycerol (MGDG) classes. The glycolipids, including MGDG were previously reported with anti-inflammatory properties [2,3], but little is known about bioactivities of betaine lipids. Moreover, these classes were abundant in esterified omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA), important precursors of anti-inflammatory mediators, some of which were already reported with anti-inflammatory activity, e.g. MGDG(34:7) and DGTS(40:10) [2,3]. Taken together these results show the high potential of polar lipids rich extracts obtained with a sustainable technique from a natural source, *C. amblystomatis*, to modulate mediators involved in the pathophysiology of inflammatory namely in skin diseases.

1. Conde, T.; Lopes, D. Metabolites 2022, 12, 96,
2. Banskota, A.H. Journal of Applied Phycology 2013, 25, 1513 -1521,
3. Banskota, A.H. Natural Product Research 2013, 27, 1084 -1090,

POSTER 19

ELEVATED ENZYMATICALLY OXIDISED PHOSPHOLIPIDS ARE DETECTED IN THE CIRCULATION IN INFLAMMATORY ARTHRITIS

DANIELA O. COSTA, Stuart T.O. Hughes, Ali A. Hajeyah, James Burston, Ana Cardus-Figueras, Robert H. Jenkins, Majd Potty, Bethan Morgan, Ernest H. Choy, Simon A. Jones, Valerie B. O'Donnell

Division of Infection and Immunity, Sir Geraint Evans Building, Heath Park, School of Medicine, Cardiff University CF14 4XN, Cardiff

Rheumatoid arthritis (RA) is linked to an increase in cardiovascular risk. RA patients have a 30% to 50% higher risk of thrombotic events, compared to the healthy population, however the mechanisms behind this are so far unknown.

Hydroxyeicosatetraenoic acid -phospholipids (HETE-PLs) are oxidized phospholipids, which can be enzymatically generated by lipoxygenases (LOXs), termed eoxPLs. They can enhance clotting through supporting PS-dependent binding and the activity of coagulation factors. Their levels are elevated in human thrombotic disorders, namely abdominal aortic aneurysm, and anti-phospholipid syndrome. To determine whether eoxPLs are elevated in arthritis-associated coagulopathies, antigen-induced arthritis (AIA) was induced in WT, Il27ra-/-, Il6ra-/- and Il6-/- mice. These develop distinct histological phenotypes similar to human histopathology - lymphoid, myeloid, fibroid (or pauci-immune) and low inflammation, respectively.

WT and Il27ra-/- AIA mice showed elevated eoxPLs, primarily 12-HETE-PEs in blood cells, as well as higher thrombin-antithrombin complexes (TAT), especially at a chronic time point. However, neither Il6ra-/- nor Il6-/- mice exhibited increased levels of TATs or eoxPLs during AIA. Chiral LC-MS/MS demonstrated 12(S)-HETE was the predominant HETE isomer in the esterified lipid pool and its increased levels were maintained upon deletion of Alox15, suggesting platelets as the primary source of these lipids. Similarly, a human RA patient study showed increased oxPLs levels in platelets, white blood cells and extracellular vesicles (EVs), compared to healthy volunteers. Furthermore, an increased plasma IgG immunological response was seen against HETE-PLs in these patients. In addition, coagulation studies revealed that EV-enriched plasma from RA patients generates more thrombin compared to healthy volunteers.

Overall, these results indicate that eoxPLs are elevated and may play a role in the increased coagulation observed in arthritis, and platelets (or platelet-derived EVs), are important players in the elevated systemic coagulation in this disease.

POSTER 20

ANTI-INFLAMMATORY 6-BROMO-INDIRUBIN-3'-GLYCEROL-OXIMETHER (6BIGOE) SELECTIVELY DOWNREGULATES COX-DERIVED LIPID MEDIATORS VIA GSK-3 INTERFERENCE IN HUMAN PRIMARY MONOCYTES

ANNA CZAPKA[1], Christian Grune[2], Patrick Schädel[1], Vivien Bachmann[1], Carlo Pergola[1], Karl Scheuer[3], Michael Dirauf[4,5], Christine Weber[4,5], Alexios Leandros Skaltsounis[6], Klaus D. Jandt[3,5], Ulrich S. Schubert[4,5], Dagmar Fischer[2,5,7] and Oliver Werz[1,5]

1) Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, 07743 Jena, Germany
2) Pharmaceutical Technology and Biopharmacy, Institute of Pharmacy, Friedrich-Schiller-University Jena, Lessingstrasse 8, 07743 Jena, Germany
3) Chair of Materials Science (CMS), Otto Schott Institute of Materials Research, Faculty of Physics & Astronomy, Friedrich-Schiller-University Jena, Jena, Germany
4) Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich-Schiller-University Jena, Humboldtstrasse 10, 07743 Jena, Germany
5) Jena Center for Soft Matter (JCSM), Friedrich-Schiller-University Jena, Philosophenweg 7, 07743 Jena, Germany
6) Department of Pharmacy, Division of Pharmacognosy and Natural Products Chemistry, University of Athens, Panepistimiopolis Zografou, GR-15771 Athens, Greece
7) Division of Pharmaceutical Technology, Department for Chemistry and Pharmacy, Friedrich Alexander University Erlangen Nürnberg, Cauerstrasse 4, 91058 Erlangen, Germany

Monocytes are innate immune cells that produce lipid mediators which are central to the initiation and progression of inflammation and can therefore be exploited as therapeutical targets. Inhibition of glycogen synthase kinase (GSK)-3 is of particular interest as it governs numerous inflammation-related processes, and differentially regulates pro- and anti-inflammatory mediators. Natural product-derived indirubins are potent GSK-3 inhibitors designed by structure-guided synthesis for improved water solubility and kinase binding. The indirubin derivative 6-bromoindirubin-3'-glycerol-oxime ether (6BIGOE) exhibits potent effects in the cellular context and inhibits 5-lipoxygenase albeit at high concentrations. Yet, the pharmacological profile regarding the metabololipidome and the mode of action of 6BIGOE in human monocytes remain unclear. Here, we show that 6BIGOE acts as a potent GSK-3 inhibitor in monocytes already at a low nanomolar range with a dualistic functionality regarding modulation of inflammatory mediators. Using a mass spectrometry-based approach, we provide comprehensive lipid mediator profiling revealing selective downregulation of cyclooxygenase (COX)-derived product formation mediated via inhibition of COX-2 expression. Other lipid mediators and precursor fatty acids were affected to a lesser extent or not at all. Via GSK-3 inhibition 6BIGOE additionally downregulates pro-inflammatory cytokines while simultaneously promoting release of anti-inflammatory IL-10. To overcome unfavorable physicochemical parameters such as high lipophilicity, 6BIGOE was encapsulated into polymer-based nanoparticles and the impact on biological functionality was assessed. Encapsulation of 6BIGOE into poly(lactic-co-glycolic) acid (PLGA)-based nanoparticles circumvented cytotoxicity at high concentrations while retaining its anti-inflammatory capacity. In ongoing experiments, we assess to which extend 6BIGOE is able to influence macrophage polarization, in particular in regard to IL-10 release and the implications for the resulting macrophage phenotype in terms of lipid mediator formation. We conclude that 6BIGOE is a dualistic modulator of inflammation, which additionally promotes a pro-resolving microenvironment. The obtained results on encapsulation of 6BIGOE mark an innovative approach for the efficient application of lipophilic indirubins and their development as therapeutical agents targeting lipid mediators.

POSTER 21

TARGETED ANALYSIS OF LIPID MEDIATORS OF INFLAMMATION IN THE STUDY OF VULNERABILITY TO POST-TRAUMATIC STRESS DISORDER (PTSD)

LAURA DESNOUVEAUX[1], Céline Dalle[1], Sandrine Livet[1], Betty Polly-Thomasson[2], Nicolas Taudon[1]*, Damien Claverie[2]*

1) IRBA - Unité de Développements Analytiques et Bioanalyse, 1 place du Général Valérie André, BP73 Brétigny sur Orge, FRANCE
2) IRBA - Unité de Neurophysiologie du Stress, 1 place du Général Valérie André, BP73 Brétigny sur Orge, FRANCE
* *Equivalent contribution*

Post-Traumatic Stress Disorder (PTSD) is a psychiatric pathology that occurs following a stressful confrontation with death. This pathology generates several extremely disabling symptoms at social, family and professional levels. Despite medical treatment, this chronic pathology remains difficult to treat and its primary prevention seems to be a better choice. For this, it is necessary to identify potential biomarkers of vulnerability. Thus, we have developed an animal model allowing an incomplete induction of PTSD, in order to study the vulnerability in mirror of the non-vulnerability.
This model mimics a predator attack. Animals were divided into two groups: stressed and controls. The study of the behavior of the stressed subjects allowed a classification into two subgroups: development vs non-development of the PTSD phenotype. The control animals have been separated into two subgroups: vulnerable or non-vulnerable, based on the frequency of an Electroencephalography wave; a biomarker previously validated (1).

Serum samples, collected at the time of sacrifice, were analyzed using a Shimadzu workpackage method (2, 3). This method allow the profile analysis for 196 lipidic mediators of inflammation. In addition to eicosanoids originating in an arachidonic acid (AA) cascade, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and other polyunsaturated-fatty-acid metabolites, these include major mediators such ethanolamide compounds, leukotrienes and platelet activating factor (PAF), as well as their metabolites. These lipidic mediators were analyzed by ultra-high performance liquid chromatography coupled to a mass spectrometer

LC40-TQ8060 (Shimadzu) using MRM mode. For a majority of the compounds, the ESI- ionization mode was used. However, the ESI+ mode was used in particular for leukotrienes and ethanolamide compounds.
Data were analyzed using SIMCA software (version XX). An OPLS-DA model was used to separate PTSD animals from non-PTSD (RÂ²Y = 0.922 and a QÂ² =0.446) by highlighting 12 lipid mediators with a correlation higher than 0.55 and lower than -0.20.

However, no model could be created to highlight potential biomarkers of this mediator's family to identify a PTSD vulnerability in the control group

(1) Claverie (2016)
(2) Hamabata (2018)
(3) Yamada (2015)

POSTER 22

LIPIDOME ANALYSES OF BRAIN AND TUMOR TISSUE IN PATIENTS WITH GLIOBLASTOMA MULTIFORME

FRANZ EGGERT[1,2], Chaoxuan Wang [1,2], Anne Pietzner[1,2], Inci Dogan[3], Michael Rothe[3], Marec v. Lehe[4] and Karsten H Weylandt[1,2]

1) Medical Department B, Division of Hepatology, Gastroenterology, Oncology, Hematology, Palliative Care, Endocrinology and Diabetes, University Hospital Ruppin-Brandenburg, Brandenburg Medical School, Neuruppin, Germany
2) Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology, Brandenburg Medical School and University of Potsdam, Potsdam, Germany
3) Lipidomix, Berlin, Germany
4) Department of Neurosurgery, University Hospital Ruppin-Brandenburg, Brandenburg Medical School, Neuruppin, Germany

The most common malignant brain tumor in adults is the wild type isocitrate dehydrogenase (IDH) glioblastoma multiforme (GBM). With a poor median survival of approximately 15 months despite surgical resection, chemo- and radiotherapy, new therapy approaches are required. It is known that beta oxidation of fatty acids (FAs) plays an important role in the energy production of GBM. The results of several studies revealed different mechanisms in GBM for FA synthesis as well as uptake from the blood. Yet it is unclear how FA uptake or synthesis contribute to substrate needs and/ or malignant behavior of the tumor.

In this study we established fatty acid measurements from glioblastoma tissue in order to better understand fatty acid and lipidome changes in GBM, in an approach to expand previous data in GBM (Martin et al. Lipids. 1996;31(12):1283-8).

Thirteen patients with histopathologically verified GBM were included, and in a first step fatty acid composition was measured using gas chromatography (GC). In correspondence with the previous data, we found differences in the fatty acid composition between nontumor (access) brain tissue and tumor tissue with decreased levels of omega-3 polyunsaturated fatty acids in tumor tissue and increased levels of omega-6 polyunsaturated fatty acids. Furthermore, in oxylipin analyses using LC-MS/MS measurements we found increased levels of a wide range of oxylipins in tumor as compared to nontumor brain tissue.

POSTER 23

HYDROXYEICOSATETRAENOIC ACIDS (HETES) LEVELS DO NOT REGULATE ENDOTHELIUM-DEPENDENT FLOW-MEDIATED DILATION OF CONDUIT ARTERIES IN HUMAN PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONDITIONS.

GUILLAUME FEUGRAY, Tony Pereira, Michele Iacob, Gaëtan Prévost, Valéry Brunel, Robisnon Joannides, Jérémy Bellien, Thomas Duflot.

Normandie Univ, UNIROUEN, INSERM U1096, CHU de Rouen, 76000 Rouen, France.

Background: We previously demonstrated that the endothelium-dependent flow-mediated dilation of conduit arteries is mainly due to the release of nitric oxide and epoxyeicosatrienoic acids and that a decreased bioavailability of these factors contribute to the endothelial dysfunction in hypertensive and type 2 diabetic patients [1]. However, one-third of the dilatory response is regulated by mechanisms that are still unknown and that can also be altered in human pathologies. This study addressed the hypothesis that the arachidonic acid-derived lipoxygenase metabolites hydroxyeicosatetraenoic acids (HETEs) are also released during flow stimulation to regulate the endothelial vasomotor function of conduit arteries in physiological and pathophysiological conditions.

Methods: This study was conducted in 16 healthy subjects, 6 essential hypertensive patients, 18 type 2 diabetic patients with hypertension and 8 diabetic subjects without hypertension. Endothelial function was assessed using radial artery flow-mediated dilation induced by hand skin heating (34 to 44°C) [1]. Local blood sampling were performed at baseline and during the maximal flow stimulation allowing to quantify plasma total (free+esterified) form of 5-, 8-, 9-, 11-, 12- and 15-HETE, according to a slightly modified validated LC-MS/MS analytical method [2].

Results: Radial artery flow-mediated dilatation in response to hand skin heating was reduced by type 2 diabetes and/or hypertension, confirming endothelial dysfunction. No difference between groups regarding HETE regioisomer concentrations was observed at baseline. Furthermore, no change in the plasma level of HETEs was observed during the endothelial stimulation in control subjects but also in patients with diabetes and/or hypertension.

Conclusions: This study shows that HETEs are not released by the endothelium of conduit arteries to regulate flow-mediated dilatation and do not contribute to the endothelial dysfunction at this level in essential hypertension and type 2 diabetes. However, further investigations focusing on the quantification of free forms of HETEs, which are thought to be the biologically active form, could be interesting to confirm these results.

1) Duflot T, Moreau-Grangé L, Roche C, et al. Altered bioavailability of epoxyeicosatrienoic acids is associated with conduit artery endothelial dysfunction in type 2 diabetic patients. Cardiovasc Diabetol. 2019;18(1):35.

2) Duflot T, Pereira T, Roche C, et al. A sensitive LC-MS/MS method for the quantification of regioisomers of epoxyeicosatrienoic and dihydroxyeicosatrienoic acids in human plasma during endothelial stimulation. Anal Bioanal Chem. 2017;409(7):1845-1855.

POSTER 24

LINOTRINS: OMEGA-3 OXYLIPINS FEATURING AN E,Z,E CONJUGATED TRIENE MOTIF ARE PRESENT IN THE PLANT KINGDOM AND ALLEVIATE INFLAMMATION IN LPS-CHALLENGED MICROGLIAL CELLS

Laurence Balas, Sujit Kumar Deya, Sophie Béraud-Dufour, Dean Edward Riechers, Olivia Augusta Landau, Justine Bertrand-Michel, THIERRY DURAND and Nicolas Blondeau

Institut des Biomolécules Max Mousseron (IBMM) UMR 5247 - CNRS - UM – ENSCM Pole Balard Recherche 1919 route de Mende F-34293 Montpellier Cedex 05 France

Alpha-linolenic acid (ALA), an essential omega-3 polyunsaturated fatty acid found in plants, exerts neuroprotection and anti-inflammatory effects in chronic and acute CNS disease models. However, the underlying mechanisms are not yet understood. Since ALA is not incorporated into the brain, the observed health benefits may result from some of its metabolites. The putative formation of dihydroxylated ALA derivatives (called linotrins) was recently shown in vitro in the presence of lipoxygenases. However, the in vitro biosynthesis of linotrins was neither stereoselective nor quantitatively efficient for studying their physiological roles as enantiomeric pure forms. Herein, we report the first stereo-controlled synthesis that features regio- and stereoselective hydrometalations of alkynes for assembling the sensitive E,Z,E-conjugated trienes, as well as LC-MS investigations that provide evidence of linotrins occurrence in plants. Moreover, strong anti- inflammatory effects on microglia highlight the potential physiological importance of linotrins and open new perspectives in search of CNS therapeutics.

POSTER 25

BRANCHED PALMITIC ACID ESTER OF HYDROXYSTEARIC ACID (PAHSA) DERIVED FROM TRIACYLGLYCEROLS ARE PRESENT IN THE BREAST MILK

Kristyna Brejchova[1], Veronika Paluchova[1,2], Marie Brezinova[1,2], Tomas Cajka[1], Laurence Balas[3], THIERRY DURAND[3], Marcela Krizova[4], Zbynek Stranak[4], Ondrej Kuda[1]

1) Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 14220 Praha 4, Czech Republic
2) First Faculty of Medicine, Charles University, Katerinska 1660/32, 12108 Praha, Czech Republic
3) Institut des Biomolecules Max Mousseron, University Montpellier, CNRS, ENSCM, Montpellier, France 4 Department of Neonatology, Institute for the Care of Mother and Child, Podolske NabrezÃ 157/36, 14700 Prague 4, Czech Republic

Breast milk is a complex mixture containing underexplored bioactive lipids. We performed an observational case-control study to compare the impact of delivery mode: caesarean section (CS) and vaginal birth (VB); and term (preterm and term delivery) on the levels of lipokines in human milk at different stages of lactation. Metabolomic analysis of the milk identified triacylglycerol estolides as a metabolic reservoir of the anti-inflammatory lipid mediator 5-palmitic acid ester of hydroxystearic acid (5-PAHSA). We found that triacylglycerol estolides were substrates of carboxyl ester lipase and 5-PAHSA-containing lipids were the least preferred substrates among tested triacylglycerol estolide isomers. This explained exceptionally high colostrum levels of 5-PAHSA in the VB group. CS and preterm birth negatively affected colostrum lipidome, including 5- PAHSA levels, but the lipidomic profiles normalized in mature milk. Mothers delivering term babies vaginally produce colostrum rich in 5-PAHSA, which could contribute to the prevention of intestinal inflammation in newborns. Keywords: human breast milk, lipidomics, PAHSA, colostrum, elective caesarean section, preterm birth

POSTER 26

NEUROPROTECTIVE EFFECTS OF 4(RS)-4-F4T-NEUROPROSTANE, A NEO-DHA, ON MICROGLIA

Xue Geng[1], Jean-Marie Galano[2], Camille Oger[2], Grace Y. Sun[3], THIERRY DURAND[2], James C. Lee[1]

1) Richard and Loan Hill Department of Biomedical Engineering, University of Illinois at Chicago, Chicago, IL 60607
2) Institut des Biomolécules Max Mousseron, IBMM,PÃ´le Chimie Balard Recherche, UMR 5247, Université de Montpellier, CNRS, ENSCM. Montpellier, France
3) Biochemistry Department, University of Missouri, Columbia, MO 65211

The abundance of docosahexaenoic acid (DHA) in brain membrane phospholipids has stimulated studies to explore its role in neurological functions. Upon released from phospholipids, DHA undergoes enzymatic reactions resulting in synthesis of bioactive docosanoids and prostanoids.

However, these phospholipids are also prone to non-enzymatic reactions leading to more complex pattern of metabolites. A non-enzymatic oxidized product of DHA, 4(RS)-4-F4t-Neuroprostane (44FNP), has been identified in cardiac and brain tissues. In this study, we examined effects of the 44FNP on oxidative and inflammatory responses in microglial cells treated with lipopolysaccharide (LPS). The 44FNP attenuated LPS-induced production of reactive oxygen species (ROS) in both primary and immortalized microglia (BV2). It also attenuated LPS-induced inflammation through suppressing NFκB-p65 and levels of iNOS and TNFalpha. In addition, 44FNP also suppressed LPS-induced mitochondrial dysfunction and upregulated the Nrf2/HO-1 antioxidative pathway. In sum, these findings with microglial cells demonstrated neuroprotective effects of this 44FNP and shed light into the potential of nutraceutical therapy for neurodegenerative diseases.

POSTER 27

DIETARY ANTIOXIDANT INTAKE IS INVERSELY ASSOCIATED WITH 2,3-DINOR OXYLIPIN METABOLITES, THE MAJOR EXCRETED OXYLIPINS IN OVERWEIGHT AND OBESE SUBJECTS

Óscar J. Lara-Guzmán[1], Diego A. Rivera[1], Vanessa Corrales-Agudelo[1], Laura Salazar-Jaramillo[1], Ángel Gil-Izquierdo[2], Sonia Medina[2], Camille Oger[3], THIERRY DURAND[3], Jean-Marie Galano[3], Juan S. Escobar[1], Katalina Muñoz-Durango[1], and Jelver A. Sierra[1]

1) Vidarium–Nutrition, Health and Wellness Research Center, Grupo Empresarial Nutresa, Calle 8 sur No. 50-67, Medellin, Colombia.

2) Research Group on Quality, Safety, and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain.

3) Institut des Biomolécules Max Mousseron (IBMM), Pôle Chimi Balard recherché, UMR 5247, CNRS, University of Montpellier, ENSCM, 1919 route de Mende 34093 Montpellier, France.

Cardiometabolic disease risk factors, including obesity, insulin resistance, high blood pressure, and dyslipidemia, are associated with elevated oxidative stress biomarkers like oxylipins. Increased adiposity by itself induces various isomers of this oxidized lipid family, while dietary polyphenols show benefits in its regulation. Previously, we showed that specific co-abundant microorganisms characterized the gut microbiota of Colombians and associated differentially with diet, lifestyle, obesity, and cardiometabolic health status, which led us to hypothesize that urinary oxylipins would reflect the intensity of oxidative metabolism linked to gut microbiota dysbiosis. Thus, we selected a convenience sample of 105 participants (age: 40.2 ± 11.9 years, 47.6% women), grouped according to microbiota, cardiometabolic health status, and body mass index (BMI); and evaluated 33 urinary oxylipins by HPLC-QqQ-MS/MS (e.g., isoprostanes, prostaglandins, and metabolites), paired with anthropometry and blood chemistry information and dietary antioxidants estimated from a 24-hour food recall. In general, oxylipins did not show differences among individuals who differed in gut microbiota. While the unmetabolized oxylipin levels were not associated with BMI, the total content of oxylipin metabolites was highest in obese and cardiometabolically abnormal subjects (e.g., insulin resistant), mainly by prostaglandin-D (2,3-dinor-11β-PGF2α) and 15-F2t-IsoPs (2,3-dinor-15-F2t-IsoP and 2,3-dinor-15-epi-15-F2t-IsoP) metabolites. The total polyphenol intake in this cohort was 1070 ± 627 mg/day. After adjusting for body weight, the polyphenol intake was significantly higher in lean than overweight and showed an inverse association with dinor-oxylipin levels in principal component analysis. These results suggest that the 2,3-dinor-oxylipins could be more specific biomarkers associated with BMI than their parent oxylipins and that are sensitive to be regulated by dietary antioxidants.

Keywords: Oxylipins, Dietary antioxidants, Biomarkers, Obesity, Oxidative Stress, Inflammation.

POSTER 28

LIPOPHENOLS: SYNTHESIS AND APPLICATIONS AS BOTH ANALYTICAL STANDARDS AND THERAPEUTICS DERIVATIVES

JORDAN LEHOUX[1], Sandra Rochel[1], Sonia Medina[2], Ángel Gil-Izquierdo[2], Maxime Vincent[3], Sylvie Begu[3], Philippe Brabet[4], THIERRY DURAND[1] and Céline Crauste[1]

1) Institut des Biomolécules Max Mousseron (IBMM), Pôle Recherche Chimie Balard, UMR5247-CNRS-UM-ENSCM,1919 rte de Mende, 34293 Montpellier, France
2) Department of Food Science and Technology, Research Group on Quality, Safety and Bioactivity of Plant Foods, CEBAS-CSIC, University Campus of Espinardo, Edif. 25, 30100 Murcia, Spain
3) Inserm U1051, Institute for Neurosciences of Montpellier (INM), France
4) Institut Charles Gerhardt (ICGM), Pole Recherche Chimie Balard, UMR5253-CNRS-UM-ENSCM, department of Advanced Materials for Catalysis and Health, 1919 rte de Mende, 34000 Montpellier, France

Lipophenols (also called phenolipids) are polyphenolic compounds acylated by a fatty acid (saturated, mono or polyunsaturated). Lipophenols have recently been identified in food matrices naturally rich in both polyphenols and fatty acids, making them natural derivatives present in human diet. The identification of natural lipophenols present in vegetables is particularly relevant to understand their pharmacological actions, metabolism or to use them as analytical standards. This study starts by the total synthesis of lipophenol derivatives which is an interesting challenge for organic chemistry due to the multiple reactive positions on polyphenols.

As an example, hydroxytyrosol (HT) linked to polyunsaturated fatty acids (PUFA) is naturally present in extra virgin olive oil and should participate to antioxidant properties of olive oil [1,2]. In the present work, chemical synthesis of HT lipophenols will be presented to access HT-PUFA standards. UHPLC MS/MS quantitative study in extra virgin olive oil was realised during a 12 months period, mimicking both commercial and inappropriate conditions of storage. The results highlighted HT-OA as a relevant marker for the monitoring of oil storage conditions and quality. The chemical synthesis of biomimetic lipophenols is also of interest to enhance the interesting properties of polyphenols (antioxidants, anticarbonyl stress action) and improve their pharmacological profile, particularly their bioavailability. Moreover, combining both therapeutic aspects of specific lipids, such as omega-3 PUFAs, and natural polyphenols in a single lipophenolic molecule is also a pharmacological strategy to obtain a synergistic potency.

Recently, we demonstrated that flavonoids-PUFA derivatives leads to the protection of retinal cells against carbony and oxidative stresses, both toxic mechanisms involved in retina dystrophy such as macular degeneration. We also showed that a proper alkyl substituent allowed to dramatically increase their anti-carbonyl stress capacity leading to strong interest on quercetine derivative bearing both an isopropyl and a PUFA part (Q-iP-DHA) [3]. In the work reported here the chemical synthesis of Q-iP- DHA allowing the access to gram scale lipophenol will be presented. Such high scale pathway was crucial to validate in vivo potency of this flavonoid lipophenol in a light induced photoreceptors degeneration mouse model.

All together, these results presented on natural and biomimetic lipophenols highlighted the importance of those new molecules in both analytical, nutrition and therapeutic purposes.

References

[1] Y. Y. Lee, C. Crauste, H. Wang, H. H. Leung, J. Vercauteren, J.-M. Galano, C. Oger, T. Durand, J. M.-F. Wan, J. C.-Y. Lee, Chem. Res. Toxicol. 2016, 29, 1689 -1698.

[2] S. Medina, R. DomÃnguez-Perles, D. AuÃ±Ã³n, E. Moine, T. Durand, C. Crauste, F. Ferreres, Ã . Gil-Izquierdo, J. Agric. Food Chem. 2020, 68, 7789 -7799.

[3] E. Moine, M. Boukhallat, D. Cia, N. Jacquemot, L. Guillou, T. Durand, J. Vercauteren, P. Brabet, C. Crauste, Free Radic.Biol. Med. 2021, 162, 367 -382.

POSTER 29

F2-DIHOMO-ISOPROSTANES AND F4-NEUROPROSTANES: PROMISING BIOMARKERS IN ALZHEIMER'S DISEASE

Ivana Milic[1], Camille Oger[2], Jean-Marie Galano[2], THIERRY DURAND[2], Andrew Devitt[1], Helen Griffiths[3], Irundika Dias[4]

1) School of Life and Health Sciences, Aston University, Birmingham, UK
2) Institut des Biomolécules Max Mousseron, UMR 5247, CNRS, UM, ENSCM, Montpellier, France
3) Swansea University Medical School, Swansea University, Swansea, UK

4) Aston Medical School, Aston University, Birmingham, UK

Alzheimers disease (AD) is a neurodegenerative disease with complex aetiology. Due to high content of polyunsaturated fatty acids (PUFA), brain is highly susceptible to free radical-mediated oxidative damage. As a result, isoprostanes (IsoP) and neuroprostanes (NeuroP) are commonly observed lipid peroxidation products in brain. However, due to low abundance, these metabolites are difficult to measure using traditional analytical tools. The aim of this work is to develop a highly sensitive and robust multiple reaction monitoring based LC-MS/MS method for the quantification of 24 different non-enzymatic F2-IsoP and F4-NeuroP and to utilise this method to analyse post-mortem brain samples from patients with AD.

This study analysed ten patients with AD and matched control frozen brain tissue samples (0.1 mg) received from Brains for Dementia, UK. Samples were homogenised in 100% methanol and spiked with internal standards (d4-4(RS)-4-F4t-NeuroP, d4-10-F4t-NeuroP and d4-10-epi-10-F4t-NeuroP). Metabolites were enriched using two-step solid phase extraction (SPE) using a polymeric SPE column (HLB PRiME, Waters) and further separation was achieved by LC-MS/MS. This assay has a linear dynamic range ($R2 > 0.93$) between 0.04ng/ml-20ng/ml for the 24 F-IsoP and F4-NeuroP. High intra- and inter-day precision ($CV < 11\%$) was observed from the QC samples.

Overall, F-IsoP and F4-NeuroP were present in higher levels in AD patient brain tissue compared to healthy subjects. Adrenic acid derived dihomo-Isofurans and docosahexaenoic acid derived F4â? NeuroP were significantly higher in AD patients compared to healthy subjects ($P<0.01$). This data with suggest that analysis of different classes of IsoP and F4-NeuroP will provide new opportunities to study lipid peroxidation in the neurodegenerative diseases such as AD.

POSTER 30

MASST SEARCH OF OXYLIPINS, LIPOPHENOLS AND LIPOPEPTIDES AS A TOOL TO SEEK ANALYTICAL AND SYNTHESIS CHALLENGES

Ángel Sánchez-Illana[1,2], Camille Oger[1], Céline Crauste[1], Claire Vigor[1], THIERRY DURAND[1], Jean-Marie Galano[1]

1) Institut des Biomolécules Max Mousseron, IBMM, Pole Chimie Balard Recherche, UMR 5247, Université de Montpellier, CNRS, ENSCM. Montpellier, France
2) Department of Analytical Chemistry, University of Valencia, Dr. Moliner 50, 46100 Burjassot, Spain.

An increasing number of novel lipid derivatives such as oxylipins, lipophenols, and lipopeptides have been synthesized and detected in biological samples. These compounds have shown bioactive and biomarker properties in different scenarios, however, the assessment of their specific distribution (including isomer distribution) among the different matrices is not always straightforward. In this regard, the re-analysis of public tandem mass spectrometry (MS/MS) datasets is a powerful tool that can help to hypothesis generation for synthesis and analytical method developments.

In this work, a MSMS-Chooser workflow and MASST tool from GNPS (https://gnps.ucsd.edu/) were employed to analyze MS/MS data from novel lipids synthesized in our laboratory including 40 oxylipins, 15 lipophenols, and 35 lipopeptides. MS/MS spectra were acquired with a flow injection method on Orbitrap system working on ESI+ and ESI- modes. Then, the MS/MS peaks were assigned, and spectra were submitted to MASST. Finally, the matches were curated and summarized.

Six different phytoprostanes and one phytofuran were the only oxylipins that shown matches, distributed mostly in plant datasets. Furthermore, it was found matches for four lipophenols derived from hydroxytyrosol, three from resveratrol, and one from quercetin. These lipophenol matches were found in human and plant datasets but this finding should be considered with caution due to the low number of MS/MS peaks in lipophenol spectra (less than 5). For lipopeptides, ten compounds matched with the public datasets.

Considering these results, we hypothesize that the limit of detection of MS/MS public datasets is above the concentration of the majority of the oxylipins. Also, we noted that most datasets are only ESI+ mode (in which the lipids are bad ionized) and the protocols employed often do not include saponification step to release the oxylipins from phospholipids. Additionally, we propose the synthesis of lipids with richer fragmentation patterns that can provide more accurate matching.

POSTER 31

LIPID PEROXIDATION PROFILING IN MARINE MICROALGAE

LINARES-MAURIZI A[1,2], Oger C[1], Galano JM[1], Balas L[1], Egea A[2], Durbec A[3], Bertrand- Michel J[3], Durand T[1], Pradelles R[2], Vigor C[1]

1) Pôle Chimie Balard Recherche, IBMM - UMR 5247, 1919, route de Mende, 34293 Montpellier, France
2) Microphyt, 713 Route de Mudaison, 34630 Baillargues, France
3) MetaboHUB, Inserm/UPS UMR 1048 - I2MC, Institut des Maladies Métaboliques et Cardiovasculaires, Toulouse, France

Microalgae are photosynthetic microscopic organisms, at the base of the food chain in aquatic environments that can synthesize a wide variety of molecules, such as polyunsaturated fatty acids (PUFAs) of the ω3 and ω6 series. Some microalgae produce high levels of ALA, EPA or DHA leading to different specific lipid peroxidation fingerprints. This oxidation degradation results from a radical or enzymatic action to form oxylipins, respectively, isoprostanoids and eicosanoids. These oxylipins known for their bioactive properties, hold great promise for many applications in the field of nutrition and wellness in particular.

Our current investigation aimed at profiling isoprostanoids and eicosanoids from four microalgae, grown under controlled conditions with an optimal medium in 10-liter photo-bioreactors. During their stationary phase, microalgae were centrifuged and the fresh biomass was harvested. A Folch extraction was performed before SPE separation. The samples were then analyzed by LC-MS/MS to determine the qualitative and quantitative profile of enzymatic and non-enzymatic oxylipins for each species.

The four selected microalgae revealed a high diversity of metabolites, up to 25 different isoprostanoids (including 5 Phytofuranes, 6 Phytoprostanes, 11 Isoprotanes and 8 Neuroprostanes), and 17 different eicosanoids (including linotrin, 1 HEPE, 2 HDHAs, protectin, resolvin, 2 HODEs, 4 HETEs, 5 EETs) present in different concentrations.
Moreover, these results shown a correlation between the most abundant PUFAs produced by different species and their oxylipins profiles.

Taken together, these findings highlight the interest of marine microalgae as a source of bioactive lipids mediators that could favour the resolution of inflammation in therapeutics or nutraceuticals. This rich mixture of oxylipins found in these marine organisms carries biological activities especially for human health benefits like antioxidant, anti-inflammatory, anti-arrhythmic, neuroprotector and also immunomodulator.

POSTER 32

MODULATORY EFFECTS OF C. ALBICANS ON LEUKOTRIENE BIOSYNTHESIS IN HUMAN NEUTROPHILS

FISCHER J., Gresnigt MS., Hube B., Werz O., Garscha U.

Nollendorfer Strasse 23, 07743 Jena / Friedrich-Ludwig-Jahn-Strasse 17, 17489 Greifswald

Candida albicans in humans is anticipated as both: a benign colonizer but also a life-threating opportunistic pathogen. As colonizer in healthy individuals it is origin of superficial mucocutaneous mycoses. However, in immunosuppressed patients, *C. albicans* can cause nosocomial life-threatening blood-stream infections. Until today, only a small number of effective anti-Candida drugs are licensed, as fungal pathogenicity mechanisms and evading strategies are only partially understood. Inflammatory processes are substantial defence mechanisms mediated by human immune cells to eradicate the pathogen and restore tissue homeostasis. As part of the general inflammatory response armoury, activated 5-lipoxygenase (5-LOX) converts free arachidonic acid (AA) to potent pro-inflammatory and chemoattractant leukotrienes (LT), which are essential in the host immune defence.

Here we investigated the capacity of *C. albicans* to trigger 5-LOX activation and LT biosynthesis as part of the host's immune defence during infection. C. albicans grows as distinct morphological forms, specified as yeast and hyphae. The latter being associated with infections and increased virulence particularly due to the cytolytic toxin Candidalysin. We found that vital C. albicans germ tubes, stimulate AA-release, LT formation and 5-LOX redistribution at the nuclear membrane of human neutrophils. Simultaneously *C. albicans* yeast cells, inactivated *C. albicans* hyphae and Candidalysin itself failed in this respect.

Deciphering the activation pathway, we identified Spleen Tyrosine Kinase (SYK) and Myeloid differentiation primary response 88 (MYD88) as essential signalling molecules for C. albicans-stimulated LT formation. Downstream activation of 5-LOX is mediated via p38 mitogen-activated protein kinases (p38 MAPK) and extracellular-signal regulated kinases 1/2 (ERK1/2), whereas Ca2+ mobilization is only from subordinate importance.

Collectively, we demonstrate that the virulent morphological of *C. albicans* (hyphae) stimulates 5-LOX activation and LT formation via SYK- and MYD88-dependent receptors and transmission of the proinflammatory signal via p38 MAPK and ERK-pathways.

POSTER 33

MAST CELLS-DERIVED LIPIDS AS MEDIATORS OF RESOLUTION OF INFLAMMATION

JOSCHUA FRIEDEL, Prof. Dr. Dr. Klaus Scholich

Friedel@med.uni-frankfurt.de Scholich@em.uni-frankfurt.de

Mast cells have the potential to play an important role in the resolution of inflammation through the synthesis and degranulation of a broad spectrum of biologically active mediators, such as interleukin (IL)-4, or prostaglandin (PG)E_2. Also, omega-3 polyunsaturated fatty acids (PUFA)-derived specialized pro-resolving mediators (SPM), such as resolvins, protectins and maresins, have been reported to actively support resolution of inflammation. Targeted screenings for cytokines, chemokines and lipid mediators known to promote resolution of inflammation showed a robust synthesis of 14(S)-hydroxy docosahexaenoic acid (14(S)-HDHA) in mast cells in response to TLR2 activation. 14(S)-HDHA is a precursor of maresin 1, a lipid probably formed by 12-lipoxygenase (12-LOX), which has been shown to promote resolution of inflammation. Accordingly, dramatic increases of 14(S)-HDHA levels were detected in inflamed tissue during resolution of zymosan, LPS or Complete Freund's adjuvant (CFA)-induced inflammation. In order to identify the potential target cells for the 14(S)-HDHA we determined the cellular neighbours of mast cells during resolution of inflammation using the MELC technology multiple sequential immunohistology. We found that during resolution of a TLR2-mediated inflammation the mast cells are located in an anti-inflammatory microenvironment, which consists of M2-like macrophages, eosinophils and dendritic cells. In addition, depletion of mast cells using Mcpt5-DTA-Cre-/+ mice resulted in a marked change in the distribution of differentiated dendritic cells within the inflammatory site suggesting that these cells are the main targets of mast cell-derived mediators.

POSTER 34

REGULATION OF 5-LIPOXYGENASE EXPRESSION AND ACTIVITY IN COLON CANCER CELLS

TAMARA GÖBEL[1], Bjarne Goebel[1], Marius Hyprath[1], Marius Mathes[1], Carlo Angioni[2], Dominique Thomas[2], Astrid S. Kahnt[1]

1) Goethe-University, Institute of Pharmaceutical Chemistry, Frankfurt/Main, Germany
2) Institute of Clinical Pharmacology, Pharmazentrum Frankfurt, University Hospital of Goethe-University, Frankfurt, Germany.

5-lipoxygenase (5-LO), mainly expressed in leukocytes, is the key enzyme in leukotriene (LT) biosynthesis and part of the initiate immune system. Its enzymatic activity is embedded in a complicated network regulating LT synthesis by various factors dependent on the cell type and nature of the stimulus in intact cells. 5-LO overexpression is very well documented in various solid cancers, among them colon cancer where it is correlated with poor prognosis regarding patient survival. In addition, 5-LO products like LTs were shown to promote the proliferation of tumor cells, but those cells were reported to release only very low amounts of LTs themselves [1]. Today, the exact role of 5-LO in cancer development and its regulation remains still not completely resolved.

A systematic approach dealing with 5-LO expression and LT biosynthesis under three dimensional (3D) cell culture has not been investigated, yet. Cancer development, as well as progression are strongly dependent on the tumor microenvironment. In monolayer culture of cancer cell lines, cell-matrix and cell-cell interactions present in native tumors are lacking. Here, cells develop artificial polarity due to cytoskeletal rearrangements as a cause of layer growth [2]. Furthermore, it is already known, that various colon cancer cell lines dysregulate several important signaling pathways due to 3D growth [3].

In the present study we aimed to investigate the expression of proteins involved in LT biosynthesis as well as LT formation of the 5-LO overexpressing colon cancer cell lines HT-29 and HCT-116. In addition, the influence of the PI3K/mTOR and MEK/ERK signaling pathways on 5-LO expression in monolayer culture was investigated.

1. Steinhilber D. et al. 5-lipoxygenase: underappreciated role of a pro-inflammatory enzyme in tumorigenesis. Front Pharmacol. 2010 Dec 24;1:143.
2. Hirschhaeuser F. et al. Multicellular tumor spheroids: an underestimated tool is catching up again. J Biotechnol. 2010 Jul 1;148(1):3-15.
3. Riedl A. et al. Comparison of cancer cells in 2D vs 3D culture reveals differences in AKT-mTOR-S6K signaling and drug responses. J Cell Sci. 2017 Jan 1;130(1):203-218.

POSTER 35

CHARACTERIZATION OF ESTERIFIED LIPID MEDIATORS, A BIOSYNTHETIC APPROACH

BJARNE GÖBEL, Dieter Steinhilber, A.K. Haefner

Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, Max-von-Laue-Str. 9, D-60438 Frankfurt/Main, Germany

Eicosanoids are an important class of lipid mediators including prostaglandins, leukotrienes and hydroperoxyl fatty acids that are formed in the arachidonic acid cascade. These mediators play a crucial role in a variety of physiological and pathophysiological processes like allergic reactions, inflammation and tumor diseases1-3. They were commonly believed to mediate their signaling actions as free acids while newer studies indicate that they also occur attached to phospholipids or glycerol. Although the signaling actions of lipid mediators are well studied today, only few information is available on their esterified counterparts. First studies demonstrate that they seem to be involved in the regulation of immune reactions and coagulation4, which might be a promising starting point for the development of new drugs. Thus, the need to investigate this new class of mediators, where a major part, concerning the effects of several esterified lipids together with their mode of action, is unknown4, arises. Unfortunately, when it comes to study these lipid mediators as well as their signaling actions, one has to overcome the fact that these compounds are either quite expensive or that they are not commercially available at all.

Therefore, we aim to establish a biosynthetic approach combining a recombinant version of the human long-chain acyl CoA synthetase 4 (ACSL4) and a human cell line overexpressing the human lysophosphatidylcholine acyltransferase 2 (LPCAT2). Using this approach, polyunsaturated fatty acids can be activated by an ACSL4 catalyzed conversion into Co-enzyme A thioesters and then transferred by LPCAT2 into lysophospholipids, in vitro. This allows a highly flexible synthesis of a large variety of phospholipids in order to facilitate the investigation of esterified lipid mediators in a more cost-efficient manner.

References
1. Schauberger, E., Peinhaupt, M., Cazares, T. & Lindsley, A.W. Curr. Allergy Asthma Rep. 16, 48 (2016).
2. Moore, G.Y. & Pidgeon, G.P. Int. J. Mol. Sci. 18, (2017).
3. Greene, E.R., Huang, S., Serhan, C.N. & Panigrahy, D. Prostaglandins Other Lipid Mediat. 96, 27 -36 (2011).
4. Thomas, C.P. et al. J. Biol. Chem. 285, 6891 -6903 (2010).

POSTER 36

INTERPLAY BETWEEN BIOACTIVE LIPID MEDIATORS AND THE GUT MICROBIOTA REGULATES INTESTINAL T CELL RESPONSES

MARIE GOEPP, Siobhan Crittenden, Chengcan Yao

The University of Edinburgh Centre for Inflammation Research, Edinburgh, UK

The gut microbiota plays a critical role in the regulation of intestinal immune responses, contributing to gut health and diseases including inflammatory bowel disease (IBD) and bowel cancer. Dysbiosis of the gut microbiota is a hallmark of IBD and cancer, which can be induced by multiple factors such as the host immune functions, genetic and epigenetic alterations, lifestyle, and environmental risk factors.

As a well-known mediator of inflammation, prostaglandin E_2 (PGE_2) is essential for the maintenance of the gut epithelial integrity and plays a critical role in tissue inflammation and bowel cancer. However, the actions of lipid mediators in gut microbiota-dependent regulation of intestinal T cells responses remain unclear. Here we report that in the steady state, blockade of biosynthesis of endogenous PGs by COX inhibition increases accumulation of regulatory T cells (Tregs) in the mouse intestine. This is reversed by activation of the PGE_2 receptor, EP4. Regulation of intestinal Tregs by PGE_2-EP4 signalling is not observed in mice treated with antibiotics, suggesting a role of the gut microbiota. 16S rRNA sequencing results suggest that PGE_2-EP4 signalling diminishes Treg-favorable commensal microbiota, and transfer of the gut microbiota that has been modified by PGE_2-EP4 signalling modulates mucosal Treg responses and exacerbates intestinal inflammation. Furthermore, PGE_2-dependent Treg inhibition depends on EP4 signalling in mononuclear phagocytes. On the other side, we have also found that PGE_2-EP4 signalling promotes pathogenic T cell responses and intestinal inflammation, which is abrogated by antibiotics treatment or by EP4 deficiency. Taken together, our results provide emergent evidence that the interaction between PGE_2 and the gut microbiota promotes intestinal inflammation through reciprocal regulation of mucosal regulatory versus inflammatory T cell responses.

Acknowledgements: We thank all our collaborators for their excellent support and collaboration.

POSTER 37

STEAROYL-COA DESATURASE-1 PROMOTES AUTOIMMUNITY BY SUPPRESSING REGULATORY T CELL DIFFERENTIATION

Elien Grajchen[1,2], Paulien Baeten[2,3], Beatriz F. CÃ´rte-Real[1,2,4], Melanie Loix[1,2], Ibrahim Hamad[1,2,4], Mansour Haidar[1,2], Jonas Dehairs[5], Jelle Y. Broos[6,7], James M. Ntambi[8], Piet Stinissen[1,2], Niels Hellings[2,3], Gijs Kooij[6], Martin Giera[7], Johannes V. Swinnen[5], Bieke Broux[2,3,9], Markus Kleinewietfeld[1,2,4], Jerome J.A. Hendriks[1,2,#,] JEROEN F.J. BOGIE[1,2,#,*]

1) Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium.
2) University MS Center Hasselt, Pelt, Belgium.
3) Neuro-Immune Connections and Repair Lab, Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium.
4) VIB Laboratory of Translational Immunomodulation, VIB Center for Inflammation Research, Hasselt University, Diepenbeek, Belgium.
5) Department of Oncology, Laboratory of Lipid Metabolism and Cancer, LKI & Leuven Cancer Institute, KU Leuven - University of Leuven, Leuven, Belgium.
6) Department of Molecular Cell Biology and Immunology, Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Amsterdam Neuroscience, MS Center Amsterdam, Amsterdam, The Netherlands.
7) Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands.
8) Department of Biochemistry, Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, USA.
9) Cardiovascular Research Institute Maastricht, Department of Internal Medicine, Maastricht University, Maastricht, The Netherlands.

*# These authors contributed equally. *Correspondence – jeroen.bogie@uhasselt.be / +32(0)11-269261)*

The imbalance between pathogenic and protective T cell subsets is a cardinal feature of autoimmune disorders such as multiple sclerosis (MS). Emerging evidence indicates that endogenous and dietary-induced changes in fatty acid metabolism have a major impact on both T cell fate and autoimmunity. To date, however, the molecular mechanisms that underlie the impact of fatty acid metabolism on T cell physiology and autoimmunity remain poorly understood. Here, we report that stearoyl-CoA desaturase-1 (SCD1), an enzyme essential for the desaturation of fatty acids and highly regulated by dietary factors, acts as an endogenous brake on regulatory T cell (Treg) differentiation and augments autoimmunity in an animal model of MS. Guided by RNA sequencing and lipidomics analysis, we found that absence of Scd1 promotes hydrolysis of triglycerides and phosphatidylcholine through adipose triglyceride lipase (ATGL). ATGL-dependent release of docosahexaenoic acid enhanced Treg differentiation by activating the nuclear receptor peroxisome proliferator-activated receptor gamma. Our findings identify fatty acid desaturation by SCD1 as an essential determinant of Treg differentiation and autoimmunity, with potentially broad implications for the development of novel therapeutic strategies and dietary interventions for autoimmune disorders.

POSTER 38

LIPOTEICHOIC ACID FROM STAPHYLOCOCCUS AUREUS DETERMINES EICOSANOID AND SPECIALIZED PRO-RESOLVING MEDIATOR PRODUCTION

KERSTIN GÜNTHER[1], Laura Miek[1], Paul M. Jordan[1], Simona Pace[1], Lorena Tuchscherr[2], Jana Gerstmeier[1], Charles N. Serhan[3], Oliver Werz[1]

1) Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller- University Jena, Jena, Germany
2) Institute of Medical Microbiology, Jena University Hospital, Jena, Germany
3) Department of Anesthesiology, Perioperative and Pain Medicine, Harvard Medical School, Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital, Boston, Massachusetts, USA

Increasing antibiotic resistance in bacteria pose a major health risk, calling for thorough investigation of the underlying molecular mechanisms. Being a reason for serious infections, *Staphyloccocus (S.) aureus* is a serious adversary in life-threatening disorders, such as sepsis or osteomyelitis. The underlying inflammatory processes are orchestrated by distinct lipid mediators (LMs) secreted from innate immune cells like macrophages, which play a major role in removal of the pathogen. These LMs comprise on one hand pro-inflammatory prostaglandins (PGs) and leukotrienes (LTs) derived from arachidonic acid via cyclooxygenases (COX) and 5-lipoxygenase (5-LOX), respectively. On the other hand, macrophages are able to produce specialized pro-resolving mediators (SPMs) derived from omega-3-fatty acids by several LOX, especially 15-lipoxygenase-1 as key enzyme. The role of LMs as well as their biosynthetic pathways in S. aureus infections remain vague.

Recently, we found that pathogenic *S. aureus* can induce LM formation in human macrophages in short-term incubations (<3 h); however, host-bacteria interactions and modulation of key LM enzyme expression and macrophage phenotypes in long-time infection are rather unknown. Here, we revealed that *S. aureus* elevates cyclooxygenase-2 and microsomal prostaglandin E2 synthase-1, but impaired the levels of 15-lipoxygenase-1, which was in line with increased PG levels and decreased SPM formation, respectively. Moreover, *S. aureus* shifts macrophages from M2-like towards an M1-like phenotype. These effects were still achieved, when heat-attenuated *S. aureus* was applied to the cells, implying that the ability to modulate LM signatures did not occur due to the vitality of *S. aureus* in long-term incubations. Furthermore, lipoteichoic acid (LTA), as one of the major constituents of the cell wall of gram-positive bacteria, mimicked the impact of *S. aureus* by massively elevating PGE_2 formation. This upregulation of COX-2 expression involves the Toll-like receptor-2/NF-kappa B - signalling axis, whereas blocking of 15-lipoxygenase-1 expression was connected to diminished levels of Lamtor1 during M2 polarization. Our results increase the knowledge about how *S. aureus* infections impact acute inflammation and resolution and will be of relevance for pharmacological interventions with *S. aureus* infections by adjusting favourable LM profiles besides or in combination with antibiotics.

POSTER 39

INFLAMMATORY MACROPHAGE MEMORY IN NSAID-EXACERBATED RESPIRATORY DISEASE

Pascal Haimerl, Ulrike Bernhardt, Sonja Schindela, Fiona Henkel, Antonie Lechner, Ulrich Zissler, Xavier Pastor, Dominique Thomas, Alexander Cecil, Yan Ge, Mark Haid, Cornelia Prehn, Janina Tokarz, Matthias Heinig, Jerzy Adamski, Carsten Schmidt-Weber, Adam Chaker, JULIA ESSER-VON BIEREN

Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Zentrum München, 80802 Munich, Germany

Background - NSAID-exacerbated respiratory disease (N-ERD) is a chronic type-2 inflammatory condition, which is driven by an aberrant arachidonic acid (AA) metabolism. Macrophages are major producers of AA metabolites and subject to metabolic reprogramming, but they have been neglected in N-ERD.

Objective - We sought to elucidate a potential metabolic and epigenetic macrophage reprogramming in N-ERD.

Methods - Transcriptional, metabolic and lipid mediator profiles in macrophages from N-ERD patients and healthy controls were assessed by RNA sequencing, Seahorse assays and LC-MS/MS. Metabolites in nasal lining fluid (NLF), sputum and plasma from N-ERD patients (n=15) and healthy individuals (n=10) were quantified by targeted metabolomics analyses. Genome-wide methylomics were deployed to define epigenetic mechanisms of macrophage reprogramming in N-ERD.

Results - We show that N-ERD monocytes/ macrophages exhibit an overall reduction in DNA methylation, aberrant metabolic profiles and an increased expression of chemokines, indicative of a persistent pro- inflammatory activation. Differentially methylated regions in N-ERD macrophages included genes involved in chemokine signaling and acylcarnitine metabolism. Acylcarnitines were increased in macrophages, sputum, NLF and plasma of N-ERD patients. Upon inflammatory challenge, N-ERD macrophages produced increased levels of acylcarnitines, pro-inflammatory AA metabolites, cytokines and chemokines as compared to healthy macrophages.

Conclusion - Together, our findings decipher a pro-inflammatory metabolic and epigenetic reprogramming of macrophages in patients suffering type-2 inflammation.

POSTER 40

ESTRADIOL AND CISPLATIN JOINT ACTION ON FEMALE RAT BRAIN NUCLEAR PHOSPHOLIPIDS CONTENT

HAKOBYAN N.R., Yavroyan Zh.V., Hovhannisyan A.G., Gevorgyan E.S.

Alex Manoogyan str.1, 0025, Yerevan, Republic of Armenia

Cisplatin (cis-diaminedichloroplatinum (II)) is widely used for the treatment of many malignancies. The efficacy of cisplatin is dose dependent, however, high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity and neurotoxicity. The results of recently investigations established that combination of steroid hormone estradiol and cisplatin in chemotherapy treatment schemes decreased cisplatin induced toxicities.

Our previous results showed the reliable changes in phospholipids in rat brain nuclear preparations after the separate in vivo action of cisplatin as well as under the alone action of estradiol.

The total amount of phospholipids and the amount of all individual lipids from rat brain nuclear preparations were decreased after the separate action of cisplatin. On the contrary estradiol, increase the total content of phospholipids up to 29%, and the amount most of individual phospholipids. Thus in case of separate action each of this drugs demonstrated its own abilities suppress or stimulate metabolic processes. Unlike these, cisplatin and estradiol combinated treatment restored the baseline level of the total amount of phospholipids and leads to peculiar summary effect in case of each individual phospholipid fraction.

Taking into consideration the dose depended active part of nuclear lipids in regulating of many essential cellular processes, it is impossible to exclude the significance of nuclear lipids quantitative alterations for reducing of cisplatin toxicity and eliminating of its side effects in case of joint use of cisplatin and estradiol.

POSTER 41

LC-ESI-MS/MS OXYLIPIN PROFILING OF HUMAN SERUM FOR STUDIES ON HYPEREMESIS GRAVIDARUM

SAIRA HAMEED, Richard Lindberg, Malin L. Nording

Dept. of Chemistry, Umeå University, Umeå, Sweden

Background: During pregnancy, the immune system adapts in order to tolerate the presence of the fetus in what can be viewed as an inflammatory state. As important regulators of inflammation, oxylipins are highly relevant to explore in relation to the pregnancy complication hyperemesis gravidarum (HG). HG is characterized by severe nausea and excessive vomiting, and affects around 2% of all pregnancies.

Objective: Oxylipins in HG have not been investigated before. The purpose of this pilot study was to apply our previously validated LC-ESI-MS/MS protocol to screen for oxylipins associated with HG at 12 weeks of gestation.

Methods: The LC-ESI-MS/MS protocol included multiple custom synthesized standards (epoxides and diols) to extend the coverage of the detected analytes. It enabled analysis of 66 oxylipins in this pilot study comparing levels between HG patients (n=3), and healthy pregnant women (n=3).

Results: Unpaired t-test showed that the Î±-linolenic acid (ALA) metabolite 15,16-dihydroxy-octadecadienoic acid (15,16-DiHODE), and the eicosapentaenoic acid (EPA) metabolite 14,15-dihydroxy-eicosatetraenoic acid (14,15-DiHETE) were lower in the serum of HG patients than those in the healthy control group ($p < 0.05$). The linoleic acid (LA) metabolite 12(13)-epoxy-9-keto-octadecenoic acid (EKODE) was higher in the serum of HG patients as compared to healthy controls ($p < 0.05$).

Conclusion: In this pilot study, three oxylipins were associated with HG. Two were decreased in serum samples collected from HG patients (the diols 15,16-DiHODE and 14,15-DiHETE derived downstream in the cytochrome P450 CYP enzymatic pathway), and one was increased (the auto-oxidative product EKODE) compared to healthy controls. This initial pilot suggests that CYP-derived oxylipins are important analytes in studies with larger sample size. Expansion of the oxylipin panel with in-house synthesized ALA-derived epoxides and diols proved useful, since 15,16-DiHODE was one of the HG-associated oxylipins that otherwise would have not been detected.

POSTER 42

GREEN, BLACK AND ROOIBOS TEA INHIBIT PROSTAGLANDIN E2 FORMATION IN HUMAN MONOCYTES BY INHIBITING EXPRESSION OF ENZYMES IN THE PROSTAGLANDIN E2 PATHWAY

ALEXANDER HEDBRANT

Alexander.hedbrant@oru.se

The use of non-steroidal anti-inflammatory drugs (NSAIDs) for long term treatment or prophylaxis of diseases and symptoms are problematic due to the risk of severe side effects. Therefore, new alternatives for inhibition of prostaglandin formation are needed. We have studied the effects of green, black and Rooibos tea extracts and selected polyphenols on the formation of prostaglandin E (PGE)2 in human primary monocytes. We found that all tea extracts and the polyphenols epigallocatechin gallate (EGCG) and quercetin inhibited the lipopolysaccharide and calcium ionophore induced PGE_2 formation in a dose-dependent manner. Green and black tea extracts inhibited PGE_2 with a similar potency and efficacy, whereas Rooibos tea extract was less potent. Further, effects of the tea extracts on the protein expression of enzymes involved in the PGE_2 synthesis pathway was studied, including cPLA2, COX-1, COX-2, and mPGES1. Inhibition of PGE_2 was shown to mainly be mediated by inhibiting the protein expression of COX-2 and mPGES-1, with the most prominent effects observed for mPGES-1. Cell-free assays were also performed to study direct effects of the tea extracts and the polyphenols EGCG and quercetin on COX and PGE synthase activity. All studied tea extracts and polyphenols induced a partial inhibition of the enzymes, observed as reduced formation of PGE_2; however, the direct inhibitory effects on the enzymes required much higher doses compared to the doses required to inhibit the protein expression of COX-2 and mPGES-1. The effects of green tea were likely mainly attributed to its EGCG content whereas other polyphenols likely are responsible for the effects observed for black and Rooibos tea. In conclusion, green and black tea, and to a lesser extent

Rooibos tea are potent inhibitors of PGE_2 formation in human monocytes and mediate their effects by inhibiting expression of enzymes in the PGE_2 synthesis pathway, especially mPGES-1.

POSTER 43

TRANSGLUTAMINASE-2 REGULATES LIPID PEROXIDATION AND MACROPHAGE FERROPTOSIS

Fiona DR Henkel, BENEDIKT SPITZLBERGER, Zhigang Rao, Franziska Hartung, Silvia de la Morte, Sonja Schindela, Sina Bohnacker, Andreas Koeberle, Julia Esser-von Bieren

Center of Allergy and Environment (ZAUM), Biedersteiner Strasse 29, 80802 Munich, Germany

Ferroptosis is a form of regulated cell death that depends on iron and lipid peroxidation. Alternatively activated M2 macrophages (AAM) play key roles in type 2 immune responses (e.g. during infection with helminth parasites) and they are particularly susceptible to lipid peroxidation and ferroptotic cell death. Here, we interrogated mechanisms that may regulate ferroptosis and thus loss of AAM in the context of helminth infection. We found abundant lipid peroxidation in the granuloma of helminth-infected mice, which correlated with high numbers of macrophages and eosinophils expressing 15-lipoxygenase, a key pro-ferroptotic enzyme. This suggested a pro-ferroptotic cellular environment in helminth infected tissues. We further observed that macrophages in the lung or peritoneal cavity of mice infected with nematode parasites upregulated transglutaminase-2 (TG2), an enzyme involved in the regulation of phospholipase activity.

Macrophages from TG2 deficient mice showed an enhanced capacity to generate lipid hydroperoxides (particularly oxPE species) and an increased PUFA/MUFA ratio in membrane phospholipids. Thus, the upregulation of TG2 in type 2 immunity may protect macrophages from lipid peroxidation and ferroptotic cell death and thereby prevent the loss of an effector cell population with essential roles in anti-helminth immunity.

POSTER 44

TARGETED ALTERATION OF THE REACTION SPECIFICITY OF LIPOXYGENASE ISOFORMS (ALOX15, ALOX15B) DURING LATE PRIMATE EVOLUTION

DAGMAR HEYDECK[1], Florian Reisch[1,2], Marjann Schäfer[1,2], Kumar R. Kakularam[1], Sophie A. Roigas[1], Sabine Stehling[1], Gerhard P. Püschel[2] and Hartmut Kuhn[1]

1) Department of Biochemistry, Charité -University Medicine Berlin, Charitéplatz 1, D-10117 Berlin, Germany.
2) Institute for Nutritional Sciences, University Potsdam, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany

Arachidonic acid lipoxygenases (ALOXs) have been implicated in the immune response of mammals. The reaction specificity of these enzymes is decisive for their biological functions and ALOX classification is based on this enzyme property. Comparing the amino acid sequences and the functional properties of selected mammalian ALOX15 orthologs we previously hypothesized that the reaction specificity of these enzymes can be predicted based on their amino acid sequences (Triad Concept) and that mammals, which are ranked in evolution below gibbons, express arachidonic acid 12-lipoxygenating ALOX15 orthologs. In contrast, Hominidae involving the great apes and humans possess 15-lipoxygenating enzymes (EvolutionaryHypothesis). These two hypotheses were based on sequence data of some 60 mammalian ALOX15 orthologs and about half of them were functionally characterized.

To put the Triad Concept and the Evolutionary Hypothesis on a broader experimental basis we compared the ALOX15 sequences of 152 mammals representing all major mammalian subclades, expressed 44 novel ALOX15 orthologs and performed extensive mutagenesis studies of their triad determinants. 23 novel Alox15 were identified from genomic sequences and the corresponding cDNA were deposited in the Third Party Annotation database. We found that ALOX15 genes are absent in extant Prototheria but that corresponding enzymes frequently occur in Metatheria and Eutheria.

More than 90% of them catalyze arachidonic acid 12-lipoxygenation and the Triad Concept is applicable to all of them. Mammals ranked in evolution above gibbons express 15-lipoxygenating ALOX15 orthologs but enzymes with similar specificity are only present in less than 5% of mammals ranked below gibbons. These mammalian species violate the Evolutionary Hypothesis. Gibbons constitute a transition taxon and the ALOX15 orthol ogs of different gibbon subspecies express ALOX15 orthologs with either pronounced dual reaction specificity or 15-lipoxygenating enzymes. Taken together, this data suggests that ALOX15 orthologs have been introduced during Prototheria-Metatheria transition and put the Triad Concept and the Evolutionary Hypothesis on a much more reliable experimental basis. Since the lipoxin and resolvin E4 synthase activity of 15-lipoxygenating ALOX15 orthologs is higher than that of 12-lipoxygenating enzymes the evolutionary switch in reaction specificity may be part of an evolutionary concept optimizing the immune system during late mammalian evolution.

POSTER 45

METABOLIC ENGINEERING OF ESCHERICHIA COLI FOR THE PRODUCTION OF HYDROXYLATED DOCOSAHEXAENOIC ACID FROM GLYCEROL

KAZUMASA HORI, Aya Miyamoto, Tetsuro Ujihara

Kyowa Hakko Bio Co., Ltd., Japan

Objective - Hydroxylated metabolites of polyunsaturated fatty acids (PUFAs) such as resolvin and protectin are called lipid mediators and act as signaling transduction molecules. From the recent studies, hydroxylated PUFAs are revealed to show various physiological properties such as anti-inflammatory, anti-infective, neuroprotective, tissue-healing activities. For further analysis, large quantities of hydroxylated PUFAs are required. However commercial scale production of these molecules have not been established. To accomplish this problem, we tried to produce hydroxylated PUFAs using genetically engineered microorganism.

Methods Used - There are several attempts for hydroxylated PUFA production by enzymatic conversion of PUFA. Enzymes such as lipoxygenase or cyclooxygenase are used in these studies. However, due to limited solubility of PUFAs to water and instability, the productivity of enzymatic conversion process could not reach to industrial demand. To overcome this problem, we tried to produce hydroxylated docosahexaenoic acid inside the bacterial cells.

Results - As a first step, we constructed docosahexaenoic acid (DHA) producing *E. coli* by introducing DHA synthesizing PUFA synthase from Aurantiochytrium sp. And next step, we introduced bacterial lipoxygenase to the strain for hydroxylation. We tried several types of lipoxygenases and found the optimal combination. The engineered strain could produce 133 mg/L hydroxylated DHA directly from glycerol. After purification, we confirmed produced hydroxylated DHA showed neuroprotective function in cultured PC12 cells.

Conclusions - We achieved to produce hydroxylated DHA directly from glucose which would be useful for functional analysis of these hydroxylated PUFAs. By applying same strategy, we can produce other hydroxylated PUFAs such as hydroxylated eicosapentaenoic acid and hydroxylated arachidonic acid by changing types of PUFA synthase and lipoxygenase.

POSTER 46

DIFFERENTIAL LIPID MEDIATOR PRODUCTION AND ENZYME EXPRESSION IN HUMAN MACROPHAGE SUBSETS

YAOLIN HOU

Division of Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet

Background Macrophages are highly versatile immune cells that play multiple roles in inflammation, including secretion of lipid mediators and cytokines. Mono Mac 6 is a widely used monocytic cell line which could be differentiated to macrophage with vitamin D3 and TGF-β.

Aims: This study aims to assess lipid mediator production in different macrophage subsets.

Methods: We isolated human peripheral blood mononuclear cells (PBMCs) and differentiate them towards M1 and M2 macrophages. As for MM6 cells differentiation, we used Vitamin D3 plus TGF-β. We analyzed lipid mediator production and related key enzyme expression in these cells. In addition, TNF-α and IL-10 expression were measured as macrophage polarization markers.

Results: M1 macrophages had a higher 5-Lipoxygenase (5-LO) expression both on mRNA and protein level, associated with abundant level of leukotriene B_4 (LTB_4) produced upon stimulation by A23187, while M2 cells expressed more 5-Lipoxygenase-1(15-LO-1). Differentiated MM6 cells gained dramatic upregulation of 5-LO expression. Upon activation with A23187, they produced abundant cysteinyl leukotrienes (CysLTs) and LTB_4, and some LXA_4 when 15-Hydroxyeicosatetraenoic acid (15(S)-HETE) was added as the substrate. qPCR analysis shows that 5-LO and 15-Lipoxygenase-2 (15-LO-2) expression were upregulated after differentiation. In addition, differentiation led to lower TNF-α and higher IL-10 expression in MM6 cells.

POSTER 47

PHARMACOKINETIC AND METABOLOMIC STUDY OF LIPID MEDIATORS AFTER CONSUMPTION OF SPMS-ENRICHED MARINE OIL IN HEALTHY SUBJECTS

Pilar Irún[1,2*], Patricia Carrera-Lasfuentes[1,2*], Marta Sánchez-Luengo[2,4], Rafael Gracia[3], María José Domper[2,4], GUSTAVO A. HIGUERA[3], Lanas Angel[1,2,4,5]

1) Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Instituto de Salud Carlos III (ISCIII), Zaragoza, Spain.

2) Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain.

3) SOLUTEX GC, SL, Zaragoza, Spain.

4) Service of Digestive Diseases, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain. 5Departamento de Medicina, Psiquiatría y Dermatología, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain.

Pro-resolving lipid mediators (SPMs) are naturally produced by immune cells by enzymatic conversion of essential fatty acids, mainly from omega-3 but also from arachidonic acid. SPMs exert potent actions on the resolution of inflammation. However, little is known about the intrinsic biorhythms of SPMs production. This study aimed to explore the 24-hour endogenous biosynthesis of omega-3 and omega-6-derived lipid mediators. A total of 23 lipid molecules were characterized in the blood of ten healthy individuals.

This is an experimental study in which 10 participants were intervened with 100ml of a marine oil enriched in SPMs. Blood samples from each subject were obtained at 0, 3, 6, 9, 12 and 24 hours for each control (standard diet) and intervention phases. Their lipid mediator metabolome was determined in plasma and serum by LC-MS/MS. Time and intervention effects on lipid mediators were performed, as well as pharmacokinetic parameters and Partial Least Squares Discriminant Analysis (PLS-DA).

Five women and five men took part in the study. The average age was 31.81 ± 5.95 years and the BMI of 23.94 ± 3.41 kg/m2. In reference to time-dependent effects, no changes were detected in the control experiment over time, but there was a time-dependent effect on SPMs after intervention.

Related to intervention-dependent effects: 1) Supplementation lead to increased plasma concentrations of w-3 fatty acids EPA and DPA at all times registered with no changes in DHA and AA concentrations versus control; 2) While 18-HEPE increased after supplementation compared to control, RvE1 production was not observed; 3) Dietary supplementation induced greater levels of PGE_2 six hours after intervention; 4) Significant increases in 14-HDHA, and 17-HDHA, precursor of protectins and resolvins were found after intervention (3-6 hours), resulting in greater concentrations of RvD1, RvD3, RvD5, Mar-2, and PDX.

With regards to pharmacokinetic parameters, the marine oil enriched in SPMs increased Cmax and AUC in EPA, 14-HDHA, 17-HDHA, and 18-HEPE together with a reduction in Tmax for 14-HDHA. Increments in Cmax and AUC were also found for DPA as it is contained by the marine oil enriched in SPMs and probably also generated by elongation of EPA. We also found higher Cmax and AUC in RvD5 and Mar-2, a higher AUC in PDX, and a reduction in Tmax of PD1 after supplementation. Results showed that the SPMs, their production, and also their pharmacokinetic parameters change noticeably between plasma and serum. Importantly, these results were confirmed by PLS-DA in both plasma and serum.

These data are a foundation to quantitatively explore modes of action of SPMs from blood, with medical benefits of Omega3´s enriched in SPMs still to be discovered.

POSTER 48

THE FLOW OF COX TO LOX STOPS AT THE RHYME; COMPREHENSIVE PROFILING REFUTES SHUNTING BETWEEN THESE PATHWAYS IN LUNG MAST CELLS

ANNA-KARIN JOHNSSON[1], Elin Rönnberg[1], David Fuchs[2], Johan Kolmert[3], Craig Wheelock[4], Gunnar Nilsson[1] and Sven-Erik Dahlèn[3]

1) Division of Immunology and Allergy, Department of Medicine Solna, Karolinska Institutet, and Karolinska University Hospital, Stockholm, Sweden
2) Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics Karolinska Institutet, Stockholm, Sweden
3) The Unit for Asthma and Allergy Research, The Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden,
4) Integrative metabolomics, The Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Lipid mediators, also termed oxylipins, are metabolites of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs). Among them, the most well-known to play an important part of mast cell biology are the prostaglandins and leukotrienes, but in theory hundreds of different lipid mediators may be synthesized from arachidonic acid and other PUFAs. A lot of what is known about mast cell lipid mediator release has been gained from animal models, however, there are large variations between species. Here we will present results from a screen of lipid mediators (in total 1153 included in the assay) secreted from human lung mast cells (HLMC) under steady state and after IgE-receptor activation. In order to examine further the enzymatic origin and interdependencies of lipid mediator production, we blocked the COX and 5-lipoxygenase pathways. This revealed that COX-1 is the predominant COX enzyme in HLMC prostanoid production, that shunting occurs within the COX-1 pathway and that the COX-1 and 5-LOX pathways are disconnected. We also show that 15-HETE, often used as a marker for 15-lipoxygenase activity, is in HLMC in fact not generated by the 15-lipoxygenase pathway. Using this large and sensitive mass spectrometry panel, we have an unbeatable tool to look at the broad and detailed picture of lipid mediator production, which can detail the flow of lipid mediators and their metabolites along their biosynthetic routes.

POSTER 49

FLAVONOID MOTIFS AS A SCAFFOLD FOR THE DEVELOPMENT OF A CYP4F-SELECTIVE OMEGA-HYDROXYLASE INHIBITOR

NADJA KAMPSCHULTE, Tim Berking, Ibrahim E. Celik, Stefan F. Kirsch, Nils Helge Schebb

School of Mathematics and Natural Sciences, University of Wuppertal, Gaussstr. 20, 42119 Wuppertal, Germany

Epoxy- and hydroxy-fatty acids (-FA) are physiologically active lipid mediators which are formed from arachidonic acid and other fatty acids by cytochrome P450 monooxygenase (CYP) catalytic activity. These oxylipins play an essential role in the regulation of blood pressure: While the effects of epoxy-FA, primarily formed by CYP2J and CYP2C, are generally vasodilative, the product of omega-hydroxylation of arachidonic acid, 20-HETE, acts vasoconstrictively in the vascular system. In contrast, in the kidney, 20-HETE exerts anti-hypertensive properties. The formation of 20-HETE in humans is dominantly catalyzed by two CYP enzymes: CYP4F2 and CYP4A11. The role of CYP4F2 and CYP4A11 in different tissues and the physiological relevance of 20-HETE formed by these isoenzymes remain to be elucidated. However, no specific CYP4A or CYP4F inhibitors have been described so far.

We recently characterized several naturally occurring flavonoids as potent CYP hydroxylase and epoxygenase inhibitors [1]. In the present study, we evaluated the structure-activity relationship of flavonoids for the inhibition of CYP-catalyzed oxylipin formation. 65 flavonoid derivatives were synthesized and tested in a natural substrate assay. We deduced structural features which lead to specific inhibition of the omega-hydroxylases, without reducing epoxygenase activity. A selective and potent CYP4F-inhibitor was found that does not inhibit CYP4A. The compound is active in human microsomes and microsomes from rodent tissues with IC50-values in the sub-micromolar range. Based on the results of pharmacokinetic characterization, we can conclude that at least moderate oral bioavailability is likely. Due to its remarkably high metabolic stability, a long half-life in vivo is expected. This novel CYP4F-selective inhibitor will allow further investigation of the role of CYP4F2 and its omega-hydroxylase activity in human physiology.

[1] Kampschulte et al. (2020), J Agric Food Chem, 68 (34) 9235-9244

POSTER 50

ADRESSING THE ACTIONS OF THE LIPID MEDIATORS 12,13-DiHOME ON PERICYTE-ENDOTHELIAL CELL JUNCTION IN THE DIABETIC HEART

SEBASTIAN KEMPF, Xiaoming Li, Stephan Klatt, Jiong Huand Ingrid Fleming

Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany.

Polyunsaturated fatty acid (PUFA) metabolites generated by the sequential action of cytochrome P450 (CYP) and soluble epoxide hydrolase (sEH) enzymes are important modulators of vascular function and integrity. Given the reported role of the sEH in regulating vascular stability in the retina, this study aimed to explore the effects of the PUFA metabolites generated by CYP/sEH on pericyte-endothelial cell junctions and to characterize the effects of sEH related PUFAs on vessel in the diabetic heart.

A comparison of wild-type mice and animals with type1 diabetes (Ins2Akita mice), revealed higher cardiomyocyte expression of the sEH in the diabetic group. The diabetic mice also displayed characteristics of heart failure with preserved ejection fraction (HFpEF) at the age of 6 months, and developed heart failure with reduced ejection fraction (HFrEF) by 12 months. Immunohistochemistry revealed a significant increase in left ventricular capillary density in 12-month-old Ins2Akita mice versus their non-diabetic littermates. However, pericyte coverage was markedly reduced in the diabetic mice. Consistent with the change in sEH expression, fatty acid profiling of the left ventricle reveled increased levels of sEH-derived PUFA diols, e.g. 12,13-dihydroxy-octadecenoic acid (12,13-DiHOME) and 14,15-dihydroxyeicosatrienoic acid (14,15-DHET) in the diabetic group. In vitro studies with 12,13-DiHOME revealed that it targeted endothelial cell-endothelial cell interactions to disrupt ba rrier function and increase permeability. Similarly, 12,13-DiHOME disrupted pericyte -endothelial cell junctions, and induce on pericyte migration.

Taken together our data indicate that the increase in sEH expression in cardiomyocytes of the diabetic heart increases the generation of 12,13-DiHOME, which disrupts endothelial cell-pericyte junctions and contributes to a decrease in vascular pericyte coverage. Studies are ongoing to determine the consequences of sEH inhibition and overexpression on pericyte coverage and vascular density in the diabetic heart.

POSTER 51

PROTEIN DISULPHIDE ISOMERASE A1 (PDIA1) INHIBITION PROTECTS AGAINST ANG II-INDUCED ENDOTHELIAL DYSFUNCTION: THE ROLE OF NO/ROS/EICOSANOIDS PATHWAYS

AGNIESZKA KIJ[1], Anna Bar[1], Izabela-Czyzynska-Cichon[1], Kamil Przyborowski[1], Bartosz Proniewski[1], Lukasz Mateuszuk[1], Agnieszka Jasztal[1], Zuzanna Kurylowicz[1], Elzbieta Buczek[1], Joanna Suraj-Prazmowska[1], Anna Kurpinska[1], Brygida Marczyk[1], Karolina Matyjaszczyk-Gwarda[1], Andreas Daiber[2], Matthias Oelze[2], Anna Tworzydlo[1], Maria Walczak[1,3], Stefan Chlopicki[1,4]

1) Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzynskiego 14, 30-348 Krakow, Poland
2) The Center for Cardiology, Department of Cardiology 1, Laboratory of Molecular Cardiology, University Medical Center, Langenbeckstr. 1, 55131, Mainz, Germany
3) Jagiellonian University Medical College, Chair and Department of Toxicology, Medyczna 9, 30-688 Krakow, Poland
4) Jagiellonian University Medical College, Chair of Pharmacology, Grzegorzecka 16, 31-531 Krakow, Poland.

Background and aim: Oxidoreductases such as protein disulphide isomerases (PDI) regulate the function of many proteins and PDIA1 inhibition results in anti-thrombotic and anti-platelet effects. However, it is not known if PDI-dependent mechanisms contribute to endothelial dysfunction and NO- and eicosanoid-dependent vascular function. Here we assessed the effects of PDIA1 inhibition on nitric oxide (NO) bioavailability, oxidative stress and vasoactive eicosanoid biosynthesis in Ang II-induced endothelial dysfunction in mice.
Methodology: Endothelial dysfunction was induced in C57BL/6 male mice by Ang II administration via osmotic pumps. To inhibit PDIA1, the solution of bepristat 2a was simultaneously administered with Ang II to selected animals. The endothelial function and vascular stiffness were assessed in vivo using magnetic resonance imaging (MRI) and pulse wave velocity (PWV) techniques, respectively. The vascular function was also measured ex vivo using myograph-based isometric tension measurements and stimulated NO aortic production by an electron paramagnetic resonance (EPR) spin trapping approach. The systemic bioavailability of NO was evaluated based on the plasma nitrite/nitrate levels and S-nitrosylhaemoglobin in erythrocytes. The ROS formation and eNOS uncoupling were assessed in aortic cryosections based on the DHE staining, while the expression of pro-inflammatory molecules was analysed immunohistochemically. The changes in eicosanoid profile were measured in plasma using ultra-p ressure liquid chromatography-triple quadrupole mass spectrometry (UPLC-MS/MS). Results and conclusions: The inhibition of PDIA1 by bepristat 2a in Ang II-treated mice improved NO-dependent vasodilation of the aorta in response to acetylcholine in vivo and ex vivo, decreased vascular stiffness and increased systemic NO bioavailability as well as aortic production of eNOS-derived NO. Moreover, PDIA1 inhibition diminished Ang II-induced overproduction of ROS, eNOS uncoupling and overexpression of pro-inflammatory vWF, however did not affect the systemic production of prostanoids and epoxyeicosatrienoic acids (EETs). Taking together, PDIA1 inhibition in Ang II-treated mice resulted in the improvement of endothelial function, suppression of vascular inflammation and remodelling that were linked to the normalisation of NO- and ROS-dependent mechanisms, but did not affect the biosynthesis of COX- and CYP450-derived eicosanoids underscoring independent regulation of NO/ROS and eicosanoids in vascular wall in the model of Ang II-induced endothelial dysfunction.

Funding: This research was funded by the Priority Research Area qLife under the program “Excellence Initiative – Research University” at the Jagiellonian University in Krakow and partially from Team Tech–Core Facility program of the FNP (Foundation for Polish Science) co-financed by the European Union under the European Regional Development Fund (project No POIR.04.04.00-00-5CAC/17–00).

POSTER 52

QUALITY EVALUATION OF N3-PUFA SUPPLEMENTS BASED ON FATTY ACID AND OXYLIPIN ANALYSIS

ELISABETH KOCH, Nadja Kampschulte, Nils Helge Schebb

Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Gaussstrasse 20, 42119 Wuppertal, Germany

Numerous studies show that a sufficient supply with the long-chain polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA, C20:5 n3) and docosahexaenoic acid (DHA, C22:6 n3) is essential for human health. It reduces the risk of coronary heart disease, enables optimal brain and visual function, and their oxidation products are believed to have anti-inflammatory effects. Thus, the German Nutrition Society and the American Heart Association among other recommend a daily intake of at least 250 mg EPA and DHA by consuming a minimum of 1-2 servings of (oily) cold water fish per week or by using n3-PUFA supplements. However, to date reports about the accuracy of EPA and DHA declaration and primary oxidation state of these supplements are inconsistent and new quality parameters beyond peroxide values are needed.

We developed a combined LC-MS based method for simultaneous quantification of precursor fatty acids and their oxidation products (eicosanoids and other oxylipins) from one sample allowing the analysis of > 200 oxylipins [Prostag Oth Lipid M 2020. 146, 106384] and 41 fatty acids [Anal Bioanal Chem 2021. 413, 5439 -5451]. Dilution of oils in iso-propanol, simple cleavage of esterified fatty acids by saponification and purification by solid phase extraction for oxylipins makes both accessible to LC-MS analysis. Eleven n3-PUFA supplements based on fish, algae and krill oil which are available over-the-counter were analyzed [J Agric Food Chem 2022. 70, 13, 3979 - 3988]. The determined EPA and DHA content was in line with manufacturer declaration - however, the overall fatty acid profile was diverse. It appears technically possible to achieve an oxidation rate as well as a non-esterified fatty acid content of < 0.1% in refined oils, whereas considerably higher values were present in unrefined krill oil. Interestingly, algae oil had high concentrations of the terminal hydroxylation products of EPA and DHA (20-HEPE, 22-HDHA), which were low in the other oils.

Our results show that LC-MS based fatty acid and oxylipin analysis i) is relevant for assessing the quality of the supplements, ii) provides information on the production of the oils as well as iii) allows an evaluation of authenticity.

POSTER 53

THE ORGANIZATION OF MICROENVIRONMENTS BY EOSINOPHIL GRANULOCYTES AND MACROPHAGES DURING LOCAL INFLAMMATION

ANJA KOLBINGER, Tim J. SchÃ¤ufele, Prof. Dr.Dr. Klaus Scholich

kolbinger@med.uni-frankfurt.de

Introduction: Pathogen-induced inflammation consists of pro-and anti-inflammatory processes, which need to ensure pathogen removal and containment of the pro-inflammatory activities simultaneously. Eosinophil granulocytes are one type of immune cell that could influence these processes by producing, storing, and releasing a great variety of pro-inflammatory and anti-inflammatory mediators, e.g., chemokines, cytokines, and lipids. This study investigates the development of spatial anti-inflammatory structures and their maintenance throughout the course of an innate toll-like receptor (TLR) 2-mediated paw inflammation and characterizes the role of eosinophils and Interleukin 4 released by eosinophils during inflammation.

Material & Methods: Local inflammation was caused through the injection of zymosan (3m/ml) in the hind paws of BL6 wild-type mice. Eosinophils were depleted with intraperitoneal injections of an anti-Siglec F antibody. Interleukin-4 was administrated as IL-4c (murine IL4+ anti-IL4). Behavior and edema formation tests showed the course of inflammation. The tissue of the mouse paws was analyzed with Flow Cytometry, Multi-Epitope-Ligand- Cartography, or Multiplex-Cytokine Assays.

Results: Within 24 hours after pathogen injection, an inflammation structure appeared, which comprised a pathogen containing core region, defined by neutrophils and pro-inflammatory M1-macrophages, a bordering M1-macrophage-containing pro-inflammatory zone, and a surrounding area with anti-inflammatory M2-macrophages. Interleukin (IL)-4 expressing eosinophils appeared early in the inflammation and were present in all three inflammatory regions. Eosinophil depletion reduced IL-4 levels and prolonged the resolution of edema formation and mechanical and thermal hypersensitivity. On the cellular level, the pro-inflammatory and antiinflammatory region became undistinguishable with increasing neutrophil numbers and decreasing efferocytosis and M2 macrophage polarization. IL-4 administration restored the inflammation structure in the eosinophil depleted mice with three different regions and normalized neutrophil numbers, efferocytosis, M2-macrophage polarization, and resolution of thermal hypersensitivity.

Conclusion: Forming a spatial organization containing a core region, a pro-inflammatory region, and an anti-inflammatory area is essential for resolving inflammation. Eosinophils support the formation through the release of Interleukin-4, which is crucial to the arrangement of the anti-inflammatory framework. Destruction of the correct zoning by eosinophil depletion affects neutrophil recruitment, efferocytosis, and macrophage polarization.

POSTER 54

EXTRACTS OF THE REGIONAL CULTIVATED CARNIVOROUS PLANT DROSERA ROTUNDIFOLIA INHIBIT 5-LIPOXYGENASE - A CHANCE FOR HUMAN AND CLIMATE PROTECTION

STEFANIE KÖNIG[1], Anne-Katrin Löser[1,2], Sandy Gerschler[2], Christian Schulze[2], Sebastian Guenther[2], Ulrike Garscha[1]

1) Chair of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, University Greifswald, Friedrich-Ludwig-Jahn Strasse 17, 17489 Greifswald, Germany
2) Chair of Pharmaceutical Biology, Institute of Pharmacy, University Greifswald, Friedrich-Ludwig-Jahn Strasse 17, 17489 Greifswald, Germany

Eicosanoids play distinguished roles in inflammatory processes and may increase or attenuate inflammatory responses, along with a major impact on cell proliferation, cell migration, phagocytosis and cytokine production. They are formed from arachidonic acid (AA) by lipoxygenases (LOXs), cyclooxygenases (COXs), and cytochrome P450 enzymes. The round leaf sundew (*Drosera rotundifolia*) is growing on nutrient-poor raised bogs and was already used in the Middle Ages to treat respiratory diseases.

However, drainage and fertilization of the peatland resulted in a significant decline of Drosera species. Currently, only Finland provides *D. rotundifolia* for wild collection but until the last years, the cultivation started in peat-exhausted moorland areas in the northeast of Germany in order to combine climate protection and pharmaceutical usage. Here, we aimed to evaluate the impact of *D. rotundifolia* extracts on leukotriene formation in human immune cells, beside the reported antibiotic effects. Extracts were prepared in house by different extracting agents gaining fractions with enrichments of various natural compound classes as flavonoids or naphthoquinones.

Different extracts of *D. rotundifolia* exhibit anti-inflammatory properties as they effectively reduce leukotriene formation by inhibition of 5-LOX. While the whole herbal extract obtained by ethanol extraction suppresses the 5-LOX product formation in Ca-ionophore-stimulated human neutrophils with an IC_{50} of 30 mikro-g/mL, extracts containing highly concentrated flavonoids such as quercetin, showed a 30-fold stronger inhibition (IC_{50} = 1 mikro-g/mL). Interestingly, naphthoquinone-containing extracts failed to improve inhibitory potency. Furthermore, extracts from international commercial Drosera herbs were inactive to hamper 5-LOX product formation in neutrophils emphasizing the influence of the extract generation.

Moreover, we confirmed a direct inactivation of 5-LOX, as recombinant 5-LOX was inhibited by the specific extracts with even higher effectiveness (whole extract: IC_{50}= 0.5 mikro-g/mL, flavonoid-rich extract: IC_{50} = 0.1 mikro-g/mL). Interestingly, extracts from regional cultivated D. rotundifolia show better inhibitory characteristics compared to extracts from commercial purchased Drosera herbs.

In summary, the extracts from carnivorous plant *D. rotundifolia* interferes directly with 5-LOX and reduced proinflammatory leukotriene formation and thus presents an interesting potential for pharmaceutical usage with simultaneous moor restoration and associated climate protection.

POSTER 55

GENETICALLY MODIFIED MICE AS RESEARCH TOOLS EXPLORING THE ROLES OF LIPOXYGENASE ISOFORM IN MOUSE MODELS OF HUMAN DISEASES.

HARTMUT KÜHN, Florian Reisch, Marjann SchÃ¤fer, Eugenia Breiträck-Marbach and Dagmar Heydeck

Department of Biochemistry, Charite-University Medicine Berlin, Chariteplatz 1, 10117 Berlin, Germany

Arachidonic acid lipoxygenase isoforms (ALOX) have been implicated in cell differentiation and programed cell death but also in the pathogenesis of inflammatory, neoplastic and neurological diseases. To explore the precise roles of these enzymes in mouse disease models knock-out and/or transgenic overexpressing mice have been developed and these animals have frequently been employed. Using the Crispr/Cas9 technology we recently developed novel genetically modified mouse strains, in which the genes encoding for Alox15, Alox15b and Alox5 were modified and these modifications altered the reaction specificities of the enzymes. The physiological and patho- physiological consequences of our genetic manipulations are currently explored in different mouse inflammation and atherosclerosis models. A brief description of the different genetically modified mouse strains is given below:

i) Alox15 knock-in mice (Alox15-KI): Mouse Alox15 is an arachidonic acid 12-lipoxygenating ALOX15 ortholog. In contrast, human ALOX15 is an arachidonic acid 15-lipoxygenating enzyme. We created mice expressing an Alox15 with humanized reaction specificity (L353F mutant) instead of the 12-lipoxygenating wildtype enzyme.

ii) Alox15b knock-in mice (ALOX15b-KI): Mouse Alox15b is an arachidonic acid 8-lipoxygenating ALOX15 ortholog. In contrast, human ALOX15B catalyzes arachidonic acid 15-lipoxygenation. We created mice expressing an Alox15b with humanized reaction specificity (Y603D+H604V mutant) instead of the 8-lipoxygenating wildtype enzyme.

iii) Alox5 knock-in mice (ALOX5-KI): Mouse and human ALOX5 exhibit similar reaction specificities. Mutation of the major triad determinants (F359W+A424I+N425M) alters the reaction specificities of both enzymes in favor of arachidonic acid 15-lipoxygenation. These mice are leukotriene deficient, produce more 13-HODE but were not protected in the DSS-colitis model.

iv) ALOX15 overexpressing transgenics (aP2-mice): Alox15-/- have frequently been used to explore the role of Alox15 in mouse disease models. Unfortunately, in most dases genetic rescue studies have not been performed. To overcome this problem, we created mice expressing human ALOX15 under the control of the aP2 promoter. This promoter directs expression of the transgene into mesenchymal cells. Including adipocytes and bone marrow cells. When crossed with Alox15-/- mice expression of the transgene rescued the hematopoietic defects induced by Alox15 deficiency.

In addition, we developed two strains of genetically modified mice (Grsf1-knock-out, Gpx4-knock-in) with indirect impact on eicosanoid biosynthesis.

POSTER 56

CONDITIONAL GRSF1 KNOCKOUT MICE DEVELOP NORMALLY BUT SHOW DISTINCT TRANSCRIPTOMIC ALTERATIONS IN THEIR TESTES

BERNHARD DUMOULIN, Moritz Lassé, Sofi Sajad, Hartmut Kühn

Charité University Medical Center, Berlin
University Medical Center Hamburg Eppendorf

The guanine-rich RNA sequence binding factor 1 (GRSF1) is an RNA-binding protein of the hnRNP H/F family and has been implicated in a variety of different cellular functions such as embryogenesis and mitochondrial RNA metabolism. Furthermore, through translational regulation of Glutathione peroxidase 4 it has been shown to play a role in regulation of lipid peroxidation. Only recently, the first in vivo study described the muscular phenotype of skeletal muscle specific Grsf1 knockout mice. To further gain insights into the role of Grsf1 in vivo we created a mouse strain that carries a compromised Grsf1 gene lacking exons 4 and 5 (Grsf1-/-) and compared the basic functional properties of these animals with those of wildtype mice. We found that Grsf1-deficient mice are viable and reproduce normally. Interestingly, we found an elevated monocyte count of Grsf1-deficient animals. However, other basic hematological parameters did not differ significantly. Female body weight kinetics of Grsf1-deficient mice were almost identical to wildtype control animals. Meanwhile, after week 15 Grsf1-deficient male mice consistently gained less weight than the corresponding control animals. We subsequently profiled mRNA concentration of Grsf1 in different tissues and found highest expression in testes. We thus performed transcriptomic analysis of testes from wildtype and Grsf1-deficient mice and GO term analysis of the differentially regulated transcripts revealed steroid biosynthesis to be most significantly upregulated. In conclusion, for the first time, we could show that conditional Grsf1 knockout mice show no major phenotypic alterations but distinct transcriptomic alterations in testicular tissue, hinting at a role for Grsf1 in steroid biosynthesis.

POSTER 57

ALLOSTERIC REGULATION OF ALOX15 AND DEVELOPMENT OF ALLOSTERIC INHIBITORS

Alexander Zhuravlev[1], Alejandro Cruz[1], Vladislav Aksenov[1], Rania Shafiullina[1], Kumar R. Kakularam[3], José M. Lluch[2], Àngels, González-Lafont[2], Igor Ivanov[1] and HARTMUT KUHN[3]

1) Lomonosov Institute of Fine Chemical Technologies, MIREA, Russian Technological University, Vernadskogo Pr. 86, 119571 Moscow, Russia
2) Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain and Institut de Biotecnologia i de Biomedicina (IBB), Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
3) Department of Biochemistry, Charite - University Medicine Berlin, Corporate member of Free University Berlin, Humboldt University Berlin and Berlin Institute of Health, Charitéplatz 1, D-10117 Berlin, Germany

Arachidonic acid lipoxygenase (ALOXs) isoforms form a family of lipid peroxidizing enzymes, which metabolize free and esterified polyunsaturated fatty acids to bioactive hormones commonly known as eicosanoids. The enzymes have been implicated in cell differentiation and maturation but also in the pathogenesis of inflammatory and metabolic diseases, such as atherosclerosis, cancer, diabetes and neurological disorders. Mammalian ALOX15 orthologs are capable of oxygenating complex lipid-protein assemblies such as biomembranes and lipoproteins and exhibit dual functionality during inflammation. Pro- and anti-inflammatory effects have been reported for these enzymes. Traditionally, ALOX isoforms have been regarded as monomeric enzymes containing a single substrate binding pocket. However, more recently, kinetic data have been published, which can only be explained when allosteric mechanisms are permitted. For the time being, the detailed mechanisms of allosteric regulation of ALOX15 orthologs remain unclear and two opposing hypotheses have been suggested: i) Existence of an allosteric regulatory center at the monomeric enzyme. ii) Dimer model in which one monomer functions as allosteric regulatory subunit. In crystals rabbit ALOX15 is present as mixed protein dimer. The two monomers (conformers A and B) have slightly different structures and interact via their Î±2 and Î±18 helices. Numerous contacts between the two helices restrain the mobility of the two monomers within the ALOX15 dimer. In aqueous solutions, the enzyme is present in a dynamic equilibrium between monomers and dimers. Mutagenesis studies, SAXS measurements and MD-simulations suggested the importance of the structural integrity of the hydrophobic intermonomer interface for ALOX15 stability and its catalytic properties. Rearrangements of inter- and intra-molecular contacts of helix 18 might impact the volume of the substrate binding pocket of the catalytic monomer in the ligand bound dimer and thus, may fine-tune the reaction specificity of the enzyme. Such intermolecular cooperativity allows modulating the activity of ALOX15 orthologs with different fatty acid substrates. We developed a set of imidazole and indole based ALOX15 inhibitors, which constitute potent inhibitors of linoleic acid oxygenation (IC50 30-40 nM), but do not impact the oxygenation of arachidonic acid at these concentrations. This substrate-specific inhibitory activity strongly suggests an allosteric mode of inhibition.

POSTER 58

FUNCTIONAL CHARACTERIZATION OF HUMAN AND MOUSE ALOX15B ORTHOLOGS AND THEIR HUMANIZED/MURINIZED ENZYME MUTANTS

KAKULARAM KUMAR REDDY, Miquel Canyelles Nino, Dagmar Heydeck and Hartmut Kuhn

Department of Biochemistry, Charite - University Medicine Berlin, Chariteplatz 1, 10117 Berlin, Germany

Abstract: Arachidonic acid lipoxygenase isoforms (ALOX) are non-heme iron-containing dioxygenases that metabolize polyunsaturated fatty acids and are widely distributed among animal and plant species including a few bacteria. Humans contain six functional ALOX isoforms (ALOX5, ALOX15, ALOX12, ALOX15B, ALOX12B, ALOXE3). The mouse genome involves a single ortholog for each human gene but in addition, an Aloxe12 gene is present, which is a corrupted pseudogene in humans. The human ALOX15B gene encodes for 15-lipoxygenase-2 that metabolizes arachidonic acid (AA) extensively to 15-hydroxyeicosatetraenoic acid (15-HETE). However, the mouse ortholog (Alox15b) produces 8-HETE and thus, this enzyme is sometimes called 8-LOX. Sequence determinants that are responsible for the distinct positional specificities of these enzyme orthologs have been explored before.

Based on this knowledge we generated a human ALOX15B mutant (D602Y+V603H) exhibiting a murinized reaction specificity. Moreover, we created a mouse Alox15b mutant (Y603D+H604V) that exhibited a humanized reaction specificity. These enzymes and the corresponding wildtype variants were expressed as N-terminal his-tag fusion proteins in E. coli and their catalytic properties were explored. As expected, the murinized human mutant and the wildtype mouse enzyme metabolized AA and EPA to the corresponding 8-hydro(pero)xy derivatives. However, with DHA as substrate the murinized human mutant produced 7-HDHA instead of 10-HDHA, which was the major DHA oxygenation product of the wildtype enzyme. The humanized mouse Alox15b and the wildtype human enzyme converted AA and EPA to the n-6 oxygenation products (15-HETE, 15-HEPE) and 17-HDHA was identified as major DHA oxygenation product. When 15-HETE and 8-HETE were used as substrates we found that wildtype mouse Alox15b rapidly metabolized 15-HETE forming 8,15-diHETE whereas 8-HETE was not oxygenated. For 15-HETE formation the substrate aligns at the active-site in tail first orientation whereas for 8-HETE the head first orientation is preferred.

Thus, for arachidonic acid oxygenation by human ALOX15B and the humanized mouse Alox15b tail-first substrate orientation was predicted. In contrast, for mouse Alox15b and murinized human mutant head-first orientation should dominate. Substrate docking studies and MD-simulations confirmed these mechanistic predictions. When head-first orientation is restricted by using phospholipids and biomembranes as substrates, 15-HETE was the dominant product of all analyzed enzyme variants.

POSTER 59

SERINE 108 OF THE SECOND CYTOSOLIC LOOP OF FLAP IS CRUCIAL FOR THE 5-LOX/FLAP COMPLEX FORMATION

KATHARINA LANDENFELD[1], Erik Romp[2], Oliver Werz[2], Ulrike Garscha[1]

1) Institute of Pharmacy, Friedrich-Ludwig-Jahn-Strasse 17 D-17489 Greifswald, Germany

2) Institute of Pharmacy Philosophenweg 14 D-07743 Jena, Germany

Leukotrienes (LT) play a pivotal role in acute and chronic inflammation. LTA_4, the precursor of LTB_4 and cysteinyl-LTs (cysLTs), is formed by oxygenation of arachidonic acid (AA) via the 5-lipoxygenase (5-LOX)-pathway. For sufficient cellular LT biosynthesis, 5-LOX translocation to the nuclear membrane and complex formation with the 5-lipoxygenase-activating protein (FLAP) is a prerequisite. Therefore, targeting FLAP is an interesting pharmacological approach to interfere with LT formation. However, to date most of the potent candidates inhibiting FLAP by competing with AA for the binding site, failed in clinical trials due to unfavourable pharmacokinetics. As the 5-LOX/FLAP complex assembly is required for LT formation, the interaction site may provide an interesting alternative target site to modulate LT biosynthesis. Here, we aimed at identifying crucial residues of FLAP that are involved in the 5-LOX/FLAP interaction. Substitutions and deletions of different FLAP residues were achieved by site-directed mutagenesis and HEK293 cell lines were generated that stably co-express 5-LOX with FLAP and its mutants, respectively. Stimulated cells were examined for LT formation, 5-LOX translocation and in situ 5-LOX/FLAP interaction by the proximity ligation assay. We found that modifications within the second cytosolic loop (C2) of FLAP implicate an altered or abolished 5-LOX/FLAP interaction. More specifically, Ser108 seems to play an important role as mutation to Ala resulted in a delayed and diminished complex formation. Substitution by Asp or Ser108-deletion even abolished 5-LOX/FLAP interaction and diminished LTA4 formation. Furthermore, in HEK cells that express the S108Î" or S108D FLAP mutant, 5-LOX product formation could not be prevented by the well-known FLAP inhibitor MK-886, which suggests a disturbed binding site for the inhibitor and possibly also for AA that mediates the 5-LOX/FLAP complex formation. As expected, 5-LOX translocation was not inhibited by all FLAP mutants.

Here, we identified Ser108 as crucial residue within the C2-region of FLAP for 5-LOX/FLAP interaction. These insights enable new strategies to design modulators that interfere with LT biosynthesis without directly competing with AA for the binding site.

POSTER 60

EFFECT OF SORAFENIB ON OMEGA-6 AND OMEGA-3 EPOXYEICOSANOID FORMATION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

CAN G LEINEWEBER[1,2], Julia Benckert[3], Jens Ricke[4], Michael Rothe[5], Nils H Schebb[6] and Karsten H Weylandt[1,2]

1) Medical Department B, Division of Hepatology, Gastroenterology, Oncology, Hematology, Palliative Care, Endocrinology and Diabetes, University Hospital Ruppin-Brandenburg, Brandenburg Medical School, Neuruppin, Germany
2) Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology, Brandenburg Medical School and University of Potsdam, Potsdam, Germany
3) Medical Department, Division of Hepatology and Gastroenterology, Campus Virchow-Klinikum, Charité, Universitätsmedizin, Berlin, Germany
4) Department of Radiology, University Hospital LMU Munich, Munich, Germany
5) Lipidomix, Berlin, Germany
6) University of Wuppertal, Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, Wuppertal, Germany

Hepatocellular carcinoma (HCC) is a leading cause of cancer death, and medical treatment options are limited. The multikinase inhibitor sorafenib was the first approved drug widely used for systemic therapy in advanced HCC. Sorafenib might affect epoxyeicosanoid levels, as it is also a potent inhibitor of the soluble epoxide hydrolase (sEH), which catalyzes the conversion of epoxides derived from long-chain polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) and omega-3 docosahexaenoic acid (DHA), into their corresponding diols. Experimental studies with AA-derived epoxyeicosatrienoic acids (EETs) have shown that they can promote tumor growth and metastasis, while DHA-derived 19,20-epoxydocosapentaenoic acid (19,20-EDP) was shown to have anti-tumor activity in mice. In a small pilot study, we found a significant increase in 11,12-EET and 14,15-EET levels in HCC patients treated with sorafenib and a trend towards increased levels of omega-3 DHA-derived 19,20-EDP. We were now able to confirm this finding, in blood samples from a large well established HCC patient population treated with sorafenib.

Furthermore, we also found increased levels of 19,20-EDP with increased blood DHA content and sorafenib treatment in these patient samples, supporting a rationale for supplementation with the n-3 PUFA DHA to increase levels of potentially beneficial 19,20-EDP in humans.

POSTER 61

COMPOUND 48/80 POTENTIATE HOUSE DUST MITE INDUCED SMOOTH MUSCLE CONTRACTION IN GUINEA PIG TRACHEA

Jielu Liu[1], Johan Kolmert[1], JESPER SÄFHOLM[1], Craig E. Wheelock[2], Sven-Erik Dahlén[1], Gunnar Nilsson[3], and Mikael Adner[1]*

1) Experimental Asthma and Allergy Research Unit, Institute of Environmental Medicine (IMM), Karolinska Institutet, Stockholm, Sweden
2) Division of Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
3) Division of Immunology and Allergy, Department of Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden.

Background and aim: Mast cells are important for allergic reactions and the development of asthma. The Mas-related G-protein coupled receptor member X2 (MRGPRX2) has been suggested to cause a non-immunological mast cell activation, however, its role during antigen-induced mast cell activation and bronchial contraction has not previously been investigated. The aim of this study was to investigate the effect of MRGPRX2 agonist on antigen induced bronchoconstriction and mediator release in house dust mite (HDM) sensitized guinea pigs.

Methods: Animals were sensitized to HDM with a single intraperitoneally injection. Tracheas were isolated two weeks later. After pretreatment with MRGPRX2 agonist (Compound (C) 48/80, 15 µg/ml, for one hour), smooth muscle contractions induced by increasing concentrations or a bolus dose of HDM were assessed in an organ bath system. Cumulatively adding of histamine to baths were performed to study the pretreatment effect on histamine H1 receptors. An unselective cyclooxygenase (COX) inhibitor (indomethacin) was presented to remove the COX-mediated guinea pig basal tone. The release of histamine and lipid mediators after HDM challenges were assessed using ELISA and mass spectrometry.

Results: Segments pre-exposed to C48/80 demonstrated increased potency (pEC50) of HDM concentration dependent response (5.1±0.1) compared to controls (4.6±0.1). Similarly, C48/80 pretreatment increased the maximal contraction of a bolus dose of HDM (0.01 µg/mL) from 14 ±6 to 47±6% of the maximal contractions. In addition, pretreatment with C48/80 caused an increase of the HDM-induced release of histamine (2-fold, p<0.05) and leukotriene (LT) B_4 (3-fold, p=0.0556) compared to HDM alone. Cysteinyl leukotrienes were not detected in neither of the groups. Furthermore, the C48/80 pretreatment reduced several of lipid mediators from arachidonic acid, α-linolenic acid, and linolenic acid.

Conclusion: Priming segments with the non-immunological mast cell agonist C48/80 potentiated the antigen induced bronchoconstriction in guinea pigs. The release data indicate that this potentiating effect is mediated by an increase release of histamine. This interaction of these two mast cell activation pathways may have implications for allergy and asthma.

POSTER 62

STRUCTURAL CHARACTERIZATION OF C-18 OXYLIPINS BY MEANS OF LC-MS AND GC-MS

ARIANE LOEWEN, Elisabeth Koch, Nils Helge Schebb

Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Gaussstrasse 20, 42119 Wuppertal, Germany

Linoleic acid (C18:2n6, LA) and Î±-linoleic acids (18:3n3, ALA) are oxidized by various enzymatic and non-enzymatic processes resulting in a complex pattern of oxylipins. While some of them have distinct biological activity such as leukotoxins, neither formation route nor structure of several 18-C oxylipins has been uncovered.

We have developed a strategy to characterize oxylipins by liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) to get information regarding the molecular formula, the position of hydroxy-group(s) and double bonds.

The characterization by LC-(ESI-)-HRMS allows the analysis of the [M-H]- ion, from which the molecular formula, and the number of oxygen atoms and double bonds can be calculated. The product ion spectrum revealed based on the Î±-cleavage next to hydroxy-groups information regarding the position of the hydroxy-group. The exact mass of the fragment ions also allows to characterize the number of double bonds within the fragment.

Analysis of the oxylipins by GC-MS after derivatization with trimethyl diazomethane and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) to the fatty acid methyl ester trimethylsilyl ether provides further information regarding the position of the hydroxyl group(s). Methylthiolation of the double bounds by iodine-catalyzed addition of dimethyl disulfide (DMDS) was used to elucidate the position of the double bond positions based on the intense fragmentation of the C-C bound between the inserted methyl sulfide groups.

With these strategy, a structural characterization of unknown oxylipin becomes possible. This is demonstrated on the poster for different mono hydroxylated 18-C-oxylipins.

POSTER 63

LIPID MEDIATOR PROFILING OF MICROGLIA

HELIKE LOHELAID, Tarvi Teder

HiLIFE/Neuroscience Center, University of Helsinki. Haartmaninkatu 8, Helsinki, Finland.

Brain resident microglia are essential in maintaining the brain homeostasis and performing immune defense. In addition, they are the first responders activated upon injury (e.g. in ischemic stroke). Defining the oxylipin lipidome of microglia and mapping the inflammatory and anti-inflammatory lipid mediators provides useful information about the lipid profile in different physiological and pathological conditions. Here we assess the activity of dioxygenases present in microglia and test different cell modulators to induce the production of various oxylipins in vitro.
In the current study, a mouse microglial cell line, BV2, was used to determine the biosynthesis of lipid mediators upon different stimuli.

Naive and IFN-gamma-polarized microglia were treated with Thapsigargin, Lipopolysaccharide (LPS) + Phorbol 12-myristate 13-acetate (PMA), calcium ionophore (A23187) or mesencephalic astrocyte-derived neurotrophic factor (MANF) for 1, 3 and 6 h. The biosynthesis of cyclooxygenase (COX) products, PGE_2, PGD_2, $PGF_2\alpha$, TXB_2, and leukotrienes (LT), LTB_4, CysLTs, were determined in BV2 cells with specific ELISA kits. In addition, biosynthesis with radiolabeled arachidonic acid (AA) was conducted with cell homogenates to determine the dioxygenase activity. Oxylipin profiles were determined by RP-HPLC/MSMS connected with DAD.
Treatment of intact BV2 cells with LPS+PMA resulted in activation of the COX pathway. The most abundant lipid mediator was PGD_2 while the levels of TXB_2, $PGF_2\alpha$ and PGE_2 remained ten folds lower. Other stimuli did not induce the production of PGs as well as LTs. At the same time, there was no difference in the production of tested eicosanoids in naive and microglia activated by INF gamma. In addition, products formed from radiolabeled AA remained undetected and further method optimization is required.

In the current study, only LPS+PMA was able to induce the production of prostaglandins in BV2 microglia. These results are in correlation with the lipid profiles of mouse macrophage-like cell line, RAW264.7 (Norris and Dennis 2012, PNAS), indicating the lower activation of 5-LOX-dependent pathways.

POSTER 64

DIFFERENTIAL THROMBOGENESIS EFFECTS OF EICOSAPENTAENOIC ACID AND DOCOSAHEXAENOIC ACID MEDIATED BY HDL PARTICLE

Honglei Li, Karthik Dhanabalan, Yanshuang Li, Jiyu Li, Connor William Dennewitz, Jiansheng Huang, Matthew Yao and WENLIANG SONG

2220 Pierce Avenue 359 Preston Research Building Nashville, TN 37232-6300

Epidemiological and observational studies have shown that consumption of marine omega-3 polyunsaturated fatty acids (n-3 PUFAs) is associated with lower cardiovascular risk. However, interventional clinical trials aimed at reducing cardiovascular incidents by supplementation of n-3 PUFAs have yielded inconsistent results. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) likely have differential TG independent effects in the cardiovascular system, which may be responsible for the disparate clinical observations. In healthy volunteers that are supplemented with EPA-enriched or DHA-enriched n-3 PUFAs, EPA was found to be much more effectively incorporated into HDL particle phospholipids compared to DHA. EPA-enriched HDL particles and DHA-enriched HDL particles have similar anti-inflammation and cholesterol efflux functions. However, EPA-enriched HDL particles have stronger anti-platelet function than the DHA-enriched HDL particles. Furthermore, EPA but not DHA substantially alleviated accelerated thrombogenesis in mice fed with a high-fat diet. This differential effect is possibly mediated by differential HDL-mediated production of specialized pro-resolving mediators (SPMs) that are derived from EPA or DHA. Although almost all of SPMs have pro-resolving effects on inflammation, different SPMs may differentially regulate platelet function. Resolvin E1 derived from EPA reduces adenosine diphosphate (ADP) activation and aggregation. However, Maresin 1 and the precursor of Resolvin Ds, 17-HDHA, both derived from DHA, significantly enhance platelet activation and aggregation. These findings provided insights for inconsistent outcome results, as well as the rationale for optimized n-3 PUFAs supplementation for various human diseases and conditions.

POSTER 65

SOLUBLE EPOXIDE HYDROLASE IS REQUIRED IN MEDIATING RESOLUTION OF INFLAMMATION

XIAOMING LI, Sebastian Kempf, Fredy Delgado Lagos, Andreas Weigert, Jiong Hu, Ingrid Fleming

Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany.

Background: Polyunsaturated fatty acids (PUFAs) play essential roles in mediating inflammation and its resolution. PUFA metabolites generated by the cytochrome P450 (CYP) - soluble epoxide hydrolase (sEH) axis are known to regulate macrophage activation/polarization but little is known about their role in the resolution of inflammation.

Methods: Monocytes were isolated from murine bone marrow or human peripheral blood and differentiated to naive macrophages (M0). Thereafter cells were polarized using LPS and IFNgamma (M1), IL-4 (M2a), or TGFbeta1 (M2c). Gene expression was analyzed by RNA sequencing, RT-qPCR and Western blotting. Phagocytosis of zymosan and oxo-LDL were also assessed in vitro. Zymosan-induced peritonitis combined with immune cell profiling was used to evaluate the resolution of inflammation in vivo.

Results: The expression of sEH was comparable in M0, M1 and M2a macrophages but markedly elevated in M2c polarized cells. The increase in sEH expression elicited by TGF-beta relied on the TGF-beta receptor ALK5 and the phosphorylation of SMAD2, which was able to bind to the sEH promoter. In macrophages lacking sEH, M2c polarization was incomplete and characterized by lower levels of pro-resolving phagocytosis associated receptors (Tlr2 and Mrc1), as well as higher levels of the pro-inflammatory markers; Nlrp3, IL-1beta and TNFalpha. Fitting with the failure to upregulate phagocytosis associated receptors, the uptake of zymosan and ox-LDL was less efficient in M2c macrophages from sEH-/- mice. The latter animals also demonstrated a retarded resolution of inflammation (zymosan-induced peritonitis) in vivo with fewer resident macrophages and recruited macrophages. PUFA profile analysis indicated decreased sEH substrates e.g., 11,12-EET, as well as increased sEH products e.g., 11,12-DHET, indicating an increased sEH activity in M2c macrophages. At the molecular level, 11,12-EET contributed to impaired M2c macrophage polarization by activating its associated receptor.

Conclusions: Taken together, our data indicates that sEH expression is required for the effective M2c polarization of macrophages and thus the resolution of inflammation.

Keywords: sEH, TGF-beta1, resolution of inflammation, PUFAs, Phagocytosis, 11,12-EET.

POSTER 66

COX-2, MPGES1 AND EP RECEPTORS EXPRESSION AND SMOOTH MUSCLE CELLS REACTIVITY IN HUMAN BRONCHI IN COPD PATIENTS

SALMA MANI[1], Dan Longrois[1,2], Xavier Norel[1]

1) Université Paris Cité and Université Sorbonne Paris Nord, INSERM, LVTS, F-75018 Paris, France.
2) Hôpital Bichat-Claude Bernard, AP-HP, Université de Paris, Paris, France.

Abstract: The PGE2 is defined as an inflammatory mediator with a bronchodilatory effect mediated by EP4 receptor. We hypothesized that a deregulated expression of COX-2, mPGES1 and EP receptors may contribute to lung function decline and changes in bronchus smooth muscle cells relaxation in chronic obstructive pulmonary disease patients (COPD).

PGE2 synthesis enzymes (COX-2 and mPGES1) and EP receptors expression in human bronchi from control subjects and patients suffering from COPD were assessed by RT-PCR, western blot and immunohistochemistry. We have investigated changes in bronchial smooth muscle cells tone in response to muscarinic agonist (carbachol) and PGE2 were evaluated using isolated organ bath system.

In human bronchi homogenates, COX-2 and mPGES1 mRNA and protein expression were increased in COPD patients when compared to controls ($P<0.05$). EP4 protein and mRNA expression were significantly reduced in COPD group compared to controls ($P<0.05$). However, EP1, EP2 and EP3 protein expression was similar between groups ($P>0.05$).

There were no significant differences ($P>0.05$) between COPD patients and controls neither for carbachol-induced bronchus smooth muscle contraction nor for PGE2-induced bronchus smooth muscle relaxation.

Increased expression of COX-2 and mPGES1 in human bronchi isolated from COPD patients suggest the co-induction of these two enzymes by cigarette smoke to provide more PGE2 in small airways. However, reduced EP2 and EP4 expression observed in bronchi homogenates from COPD patients may compensate and maintain the bronchial tone induced by PGE2 at a similar

level as in non-COPD control preparations. On the other hand, PGE2 increased level could contribute to chronic inflammation.

POSTER 67

INFLUENCE OF SEX AND SEXUAL HORMONES ON THE METABOLOLIPIDOME OF MONOCYTE-DERIVED MACROPHAGES AND ON THEIR ADAPTATION OF FUNCTIONAL POLARIZATION STATES

KATHARINA PAULA LYDIA MEYER, Patrick Schädel, Ines Kleemeier, Vivien Bachmann, Mareike Wichmann, Oliver Werz

Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich Schiller University Jena, Philosophenweg 14, Jena 07743, Germany

Sexual dimorphisms in immune function and immunological responses account for disparities in prevalence, severity and pathogenesis in infectious diseases between women and men. Hormonal mediators play a crucial role in these discrepancies, particularly in innate immunity.

Macrophages as major effector cells of innate immune responses contribute to the defense against pathogens and maintain homeostatic clearance and wound-healing processes through the release of bioactive lipid mediators (LM), respectively. Endogenous danger signals as well as sex hormones can alter macrophage phenotypes from classical to alternative activation, modulate their maturation, and regulate immune responses. Research on sex-based differences in innate immunity gained increasing interest, however, cellular characteristics and signaling pathways underlying the sex differences in the response of macrophages to infection need further elucidation. Here we show the influence of sex and sexual hormones on the metabololipidome of human monocyte-derived macrophages (MDM) in the context of bacterial infection/stimulation. Through side-by-side comparison of pro-inflammatory (M1) and pro-resolving (M2) MDMs, stimulated with pathogenic E. coli or S. aureus, we revealed sex-specific LM signature profiles and document the influence of the sex hormones 5alpha-dihydrotestosterone, 17beta-estradiol and progesterone on the metabololipidome. Analysis of the polarization state and the phagocytic capabilities of MDMs imply distinct macrophage phenotypes after sex hormone treatment beyond the classical M1 and M2 dichotomy. Our results emphasize a multi-facetted sex-bias in the metabololipidome of human MDMs in the context of bacterial infections, highlighting the impact of sex hormones on both, MDM differentiation and polarization. Together, we identified sex disparities in the innate immune response focusing on macrophage plasticity connected to LM. These findings may advance the field to properly address sex differences in inflammation research and provide a starting point for personalized therapeutic interventions for inflammatory diseases.

POSTER 68

THE MEDIUM-CHAIN FATTY ACID - GPR84 AXIS IN SEPSIS

LINDA MOET, Peter Ghazal

25 Lock Keepers Court CF10 3EZ Cardiff

Sepsis is a serious, life-threatening condition in which the immune system response to an infection leads to multi organ failure. While the aberrant immune response is the acknowledged cause, cellular metabolism is known to be severely disrupted as well. In this study we looked at changes in lipid metabolism of saturated and poly-unsaturated fatty acids in sepsis by measuring lipids in plasma samples of adult sepsis patients and healthy controls using LC-MS. Our focus of interest is on medium-chain fatty acids that bind to the receptor GPR84, a G-protein coupled receptor significantly upregulated in sepsis. Since medium-chain fatty acids previously have only been measured in low amounts or were not detected in plasma of healthy controls, we wanted to establish if they are present in the plasma of sepsis patients. To this end we established a sensitive, targeted method to quantify these lipids using extraction, derivatisation and LC-MS. Indeed, we measured medium-chain fatty acids able to bind GPR84 in the plasma of a subset of sepsis patients in sufficient amounts to activate the receptor. Other measured lipids were significantly changed between sepsis patients and healthy controls as well, including acylcarnitines and lipid mediator precursors: arachidonic acid and eicosapentaenoic acid. To further elucidate the potential role the medium-chain fatty acid - GPR84 axis plays in sepsis, the relationship between medium-chain fatty acids, other lipids and clinical measurements and was examined. Furthermore, the role of GPR84 was addressed by analysing its transcriptional regulation using an in silico approach. These findings suggest that GPR84 is upregulated early in the immune response and in response to inflammatory stimuli. Taken together, our results suggest that in a subset of sepsis patients the MCFA - GPR84 axis may amplify the innate immune response early in infection.

POSTER 69

THE SOLUBLE EPOXIDE HYDROLASE PLAYS A KEY ROLE IN MODULATING EPIDERMAL HOMEOSTASIS AND SKIN INFLAMMATION

ZUMER NAEEM, Sven Zukunft, Arnaud Huard, Andreas Weigert, Bruce D. Hammock, Timo Frömel, Ingrid Fleming.

naeem@vrc.uni-frankfurt.de

Cytochrome P450 (CYP) enzymes are membrane bound, heme containing terminal oxidases that metabolize endogenous poly-unsaturated fatty acids (PUFAs) to their corresponding epoxides, generating bioactive lipid mediators that have been attributed anti-inflammatory properties.These bioactive epoxides are further metabolized by the soluble epoxide hydrolase (sEH) to corresponding less active diols.

PUFA metabolism is highly active in the skin but little is known about the role of the CYP-sEH axis in this organ. LC-MS/MS based free fatty acid analyses revealed that dorsal skin from wild-type mice is enriched in sEH-derived PUFA diols, that were lacking in skin from sEH-/- mice.

Phenotypically, the proliferation of basal keratinocytes was greater in sEH-deficient mice, which also demonstrated thicker differentiated spinous and corneocyte layers than wild-type mice. In the latter animals, the topical application of a sEH inhibitor induced a similar hyperkeratosis phenotype. Although the inhibition of the sEH is generally associated with anti-inflammatory effects, sEH deletion made skin more prone to inflammation triggered by mechanical as well as to chemical stress.
Mechanical stress induce by depilation (milder stress) or stripping (severe stress) resulted in pronounced skin irritation and the infiltration of immune cells including neutrophils, Langerhans cells, monocytes and macrophages within 24 hours of depilation of sEH-/- mice. sEH-/- mice were also more sensitive to Imiquimod-induced psoriasis and developed thicker psoriasis plaques compared to the control group. This phenomenon was also associated with increased neutrophil infiltration and was coincident with an increase in 12,13-epoxyoctadecenoic acid (EpOME) and leukotrine B_4 (LTB_4). Both 12,13-EpOME and LTB4 have been reported to attract and activate neutrophils in the skin of burn victims. Ex vivo studies revealed that 12,13-EpOME significantly increased neutrophil adhesion and neutrophil elastase activity.

In summary, the sEH is highly active in murine skin and its deletion alters the abundance of local fatty acids in different skin layers, which has consequences on keratinocyte proliferation and differentiation in the epidermis. The deletion of the sEH resulted in an exaggerated immune response during atopic dermatitis which was partially attributed to altered fatty acid profile, suggesting that this may be an important side effect of sEH inhibitor therapy.

POSTER 70

ETHANOL + PLD = PHOSPHATIDYLETHANOL, A LONG-TERM ALCOHOL BIOMARKER

PAUL O. NEILSEN, L. Jeff Johnson, Piotr W. Rzepecki, Sean S. Davies

Echelon Biosciences, Salt Lake City, UT Vanderbilt University, Nashville, TN United States of America

Problem drinking is a major health problem and accurate biomarkers are needed to report cumulative intake, drinking patterns, as well as alcohol-induced organ damage. Traditional indirect markers of alcohol ingestion, including the percent of transferrin with improper glycosylation (%CDT), have certain limitations; so direct biomarkers Phosphatidylethanol (PEth), ethyl glucuronide (EtG) and ethylsulfate (EtS) are gaining increased use and acceptance. The effect of drinking patterns on these non-oxidative ethanol metabolites is not well understood. We enrolled 30 social drinkers in a controlled drinking study to evaluate and compare biomarkers for ethanol consumption. Two groups of drinkers (binge and consistent) consumed similar amounts of alcohol for one week with three weeks of abstinence both before and after drinking week. We sampled urine for EtS/EtG, serum for %CDT, and blood for PEth; and monitored drinking compliance by ankle bracelet (SCRAM for Secure Continuous Remote Alcohol Monitoring). We found that for both patterns, direct biomarkers were superior to indirect biomarkers for detecting alcohol consumption at 1, 7 and 21 days-post drinking. We also observed that the SCRAM bracelets helped to deter and detect off-protocol drinking even if they were not perfectly sensitive.

POSTER 71

OXYLIPIN PROFILING BY LC-ESI-MS/MS IN CANINE SERUM, PLASMA AND URINE TO IDENTIFY BIOMARKERS FOR OVULATION AND PREGNANCY IN DOGS

MALIN L. NORDING[1], Margareta Ramström[1], Bodil S. Holst[2]

1) Dept. of Chemistry, Umeå University, Umeå, Sweden
2) Dept. of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Pregnancy is an inflammatory state during which the immune system adapts in order to tolerate the presence of the fetus. These immune system adaptations change the levels of certain oxylipins that can be detected by LC-ESI-MS/MS, but it is unknown to which extent in bitches. By monitoring oxylipin profiles during the reproductive cycle of the domestic bitch, putative biomarkers for ovulation and pregnancy may be identified. To that end, canine serum, plasma and urine were analyzed for oxylipin profiles for the long-term goal of developing non-invasive and early diagnosis of canine pregnancy and of ovulation.

Methods: Using our previously validated LC-ESI-MS/MS protocol for oxylipin quantification, 66 oxylipins were probed for in canine serum (n=6), plasma (n=6), and urine (n=3) to investigate oxylipin profiles in different sample types. As a pilot study, the same analytical protocol was used for analyzing serum samples collected from bitches before ovulation (n=10), during ovulation (n=10), and after ovulation (n=10). The day of ovulation was estimated based on serum progesterone concentrations.

Results: The method was successfully applied to all three sample types. The largest number of oxylipins was detected in serum (up to 51) compared to plasma (up to 47) and urine (up to 24). In the pilot study, nine oxylipins (Resolvin D1, 20-HETE, 5-HEPE, 7,8-DiHDPE, 12,13-DiHODE, 8-HETE, 17-HDoHE, and PGE2) were detected at levels that differed significantly ($p<0.1$) from those at ovulation, either before or after ovulation.

Conclusions: The LC-ESI-MS/MS method employed for oxylipin profiling was able to detect a large array of oxylipins in canine serum and plasma. Serum samples collected at different time points during the canine reproductive cycle could be discriminated based on their levels of nine oxylipins. The method needs further development and validation for application to urine samples before being used in larger studies on bigger cohorts of dogs.

POSTER 72

PRO-RESOLVING LIPID MEDIATORS IN ALZHEIMER'S DISEASE

MAKIKO OHSHIMA[1], Ceren Emre[1], Nicolas G. Bazan[2], Marianne Schultzberg[1]

1) Karolinska Institutet, Stockholm, Sweden
2) Louisiana State University Health New Orleans, New Orleans, USA

Detrimental chronic inflammation drives Alzheimer's disease (AD) pathology, indicating that the resolution process that terminates inflammation is impaired in AD. The resolution is mediated by lipid mediators (LMs), so called specialized pro-resolving lipid mediators (SPMs) including lipoxins (LX), maresins (MaR), resolvins (Rv) and protectins (PD) which are produced from the poly-unsaturated fatty acids (FAs) omega-3 (n-3) and n-6 FAs, and act on G-protein-coupled receptors and peroxisome proliferator-activated receptors (PPAR). We have shown reduced levels of SPMs such as LXA_4 and MaR1, and altered levels of receptors for SPMs in the brain in AD, indicating a dysfunctional resolution that may lead to the chronic inflammation in the AD brain. Recent data show reduced levels of SPMs and increased levels of pro-inflammatory lipids in cerebrospinal fluid (CSF) samples from patients with AD and mild cognitive impairment compared to cases with subjective cognitive impairment (SCI). Our in vitro studies show that SPMs stimulate amyloid β (Aβ) phagocytosis by microglia and reduce Aβ-induced pro-inflammatory phenotype, NF-κB activation and NLRP3-inflammasome activation in microglia, as well as reduce neuronal cell death, all of which conceivably would be beneficial in the brain in AD.

We have investigated the effects of SPMs in a mouse AD model based on knock-in of humanised amyloid precursor protein (APP) (AppNL-G-F homozygotic mice) and showed that intranasal administration of a mixture of SPMs improved cognitive function and decreased neuroinflammation in the brain in 6-month-old mice. Furthermore, the treatment restored gamma oscillation deficits. The data support that targeting the dysfunctional resolution of inflammation in AD is a potential future treatment option.

The support by The Swedish Research Council, The Swedish Alzheimer Foundation, The Swedish Brain Foundation, Åhlén Foundation and Dementia Foundation, is gratefully acknowledged.

POSTER 73

SPHINGOSINE 1-PHOSPHATE RECEPTOR 5 (S1P5) KNOCKOUT AMELIORATES ADENINE-INDUCED NEPHROPATHY

SAMMY PATYNA, Timon Eckes, Alexander Koch, Anke Oftring, Stefan Gauer, Nicholas ObermÜller, Stephanie Schwalm, Liliana Schaefer, Jerold Chun, Hermann-Josef Gröne and Josef Pfeilschifter

Institute of General Pharmacology and Toxicology, University Hospital Frankfurt, Goethe University Frankfurt am Main, 60590 Frankfurt am Main, Germany

S1P and its receptors have been reported to play important roles in the development of renal fibrosis. Although S1P5 has barely been investigated so far, there are indications that it can influence inflammatory and fibrotic processes. Here, we report the role of S1P5 in renal inflammation and fibrosis. Male S1P5 knockout mice and wild-type mice on a C57BL/6J background were fed with an adenine-rich diet for 7 days or 14 days to induce tubulointerstitial fibrosis. The kidneys of untreated mice served as respective controls. Kidney damage, fibrosis, and inflammation in kidney tissues were analyzed by real-time PCR, Western blot, and histological staining. Renal function was assessed by plasma creatinine ELISA. The S1P5 knockout mice had better renal function and showed less kidney damage, less proinflammatory cytokine release, and less fibrosis after 7 days and 14 days of an adenine-rich diet compared to wild-type mice. S1P5 knockout ameliorates tubular damage and tubulointerstitial fibrosis in a model of adenine-induced nephropathy in mice. Thus, targeting S1P5 might be a promising goal for the pharmacological treatment of kidney diseases.

POSTER 74

SET UP AND VALIDATION OF A QUANTIFICATION METHOD FOR COX METABOLITES OF ARACHIDONIC ACID AND ENDOCANNABINOIDS

ADRIEN PAQUOT, Giulio G. Muccioli

Av. E. Mounier 72, B1 72.01 1200 Brussels, Belgium

The sequential metabolism of arachidonic acid by cyclooxygenases (COXs) and prostaglandin synthases results in the production of the well -characterized prostaglandins. The seminal work by the group of Marnett demonstrated that the endocannabinoids 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (anandamide, AEA) follow the same pathway resulting in the production of two new families of lipid mediators namely the prostaglandin-glycerol esters (PG-G) and prostaglandin-ethanolamides (PG-EA). While their biosynthetic pathway is well characterized, there is still a knowledge gap regarding their biological properties and roles. For some of them a role in the modulation of inflammation has been put forth. However, it is still unclear if these compounds play roles in early or late inflammatory phases or if they have physiological roles besides inflammation as reported for prostaglandins. To push the field forward, there is a need for quantification methods allowing to quantify their levels in vitro and in vivo. For those reasons, we developed a quantification method allowing for the analysis of the classical prostaglandins as well as their glycerol (i.e. PG-G) and ethanolamide (i.e. PG-EA) derivatives. One of the main challenges in their analysis is the low endogenous abundance of some of these lipid mediators. We therefore decided to use a tandem quadrupole to develop a UPLC-MS/MS - based method. We describe here the different steps leading to the validation of a sensitive method. We first developed a liquid-liquid extraction followed by a purification on SPE columns. The extract was then analysed on an Acquity UPLC class H - Xevo TQ-S instrument (Waters). An Acquity BEH C18 column was selected to efficiently resolve the isomers (i.e. PGD2 and PGE2 series). A gradient was optimized using water and acetonitrile containing acetic acid as mobile phases. An ESI source operating in positive ionization mode was used and the MRM transitions were determined using the optimized analytical conditions we selected. Due to the low endogenous abundance of the PG-G and PG-EA in biological matrices we aimed for limits of detection in the order of fmol/column. Altogether these steps should allow us to detect several prostaglandin derivatives in biological matrices.

POSTER 75

CANNABIDIOL ACTIVATES HUMAN MACROPHAGES TO PRODUCE SPECIALIZED PRO-RESOLVING MEDIATORS (SPMS)

LUKAS K. PELTNER[1], Lars Gluthmann[1], Rossella Bilancia[2], Simona Pace[1], Antonietta Rossi[2], Paul M. Jordan[1], Oliver Werz[1]

1) Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, 07743 Jena, Germany
2) Rossella Bilancia, Antonietta Rossi (Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy

The plant *Cannabis sativa* and its constituents have been used as an anti-inflammatory remedy for centuries. As one of the most abundant active substances in *Cannabis sativa*, cannabidiol (CBD) has recently been discussed as treatment for a large number of inflammatory diseases such as Chrons disease, ulcerative colitis, and inflammatory skin diseases. CBD and other cannabinoids exert pleiotropic effects on several immune cells, but how they influence lipid mediator (LM) formation in innate immune cells is still elusive. Human monocyte-derived macrophages (MDM) orchestrate the inflammatory response through production of a broad spectrum of bioactive lipid mediators and were thus employed as suitable test system in this study. Bioactivity screening of various cannabinoids contained in *Cannabis sativa* revealed cannabidiol (CBD) as the most potent substance regarding the ability to stimulate the production of specialized pro-resolving mediators (SPMs) and SPM precursors while simultaneously inhibiting 5-lipoxygenase (LOX) product formation. Upon investigating how CBD stimulates SPM production, we found that CBD exerts these effects by liberating fatty acids through cytosolic phospholipase A2 (cPLA2) and calcium-independent activation of 15-LOX-1. We further reveal that CBD activates both 15-LOX-1 and -2 in cell-based assays using M2 macrophages and HEK293 transfected with various LOXs. Similar to macrophages, we also found that neutrophils and platelets synthesize high amounts of SPM precursors in the presence of CBD. Furthermore, by administration of CBD to mice in a zymosan-induced peritonitis in vivo model, we found enhanced SPM and 12-LOX product levels in the peritoneal cavity, while 5-LOX and COX product formation remained unchanged.

Conclusively, this suggests that the anti-inflammatory actions of Cannabis sativa might be based, in part, on the effects of cannabinoids (*i.e.* CBD) on the production of SPMs and suppression of pro-inflammatory lipid mediators. Further research into the mechanisms may help to enhance treatment strategies and to foster the development of new drugs exploiting these pathways.

POSTER 76

ALTERED LIPID MEDIATOR PROFILE IN TYPE 1 DIABETES: DEFECTIVE RESOLUTION OF INFLAMMATION IN PANCREATIC ISLETS?

PEREZ-HERNANDEZ, JAVIER[1], Ferrer-Ferrer, Lucas[1], Salignac, Sherlina[1], Lavaud Cassandra[1], Courtalon, Camille[1], Bonnefoy, Francis[2], Perruche, Sylvain[2], Mallone, Roberto[1] and You, Sylvaine[1].

1) Université de Paris, Institut Cochin, CNRS, Institut National de la Santé et de le Recherche Médicale (INSERM), Paris, France.

2) Université Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098 RIGHT, Interactions Hôte- Greffon-Tumeur/Ingénierie Cellulaire et Génique, Fédération Hospitalo-Universitaire INCREASE, LabEx LipSTIC, Besançon, France.

Introduction: Type 1 diabetes (T1D) is a chronic autoimmune disease leading to the selective destruction of insulin-producing beta cells by autoreactive T lymphocytes. Recent evidence supports an important contribution of innate immune cells to T1D pathogenesis at early stages of the disease. In mice, islet-resident macrophages display a basal pro-inflammatory state, resembling to those present in barrier tissues. We hypothesized that resolution of inflammation is impaired in T1D, leading to beta cell death in the islets and contributing to the loss of immune tolerance and the propagation of autoimmunity.

Methods: To identify different pro-inflammatory / specialized pro-resolving mediator (SPM) profiles associated to T1D, we have performed plasma lipidomics from healthy (n=25) and T1D subjects (n=25). For mouse lipidomics, we have used a spontaneous and progressive model of T1D (NOD mouse) and quantified lipid mediator levels for both, plasma and pancreas, at different stages of the disease (young, young pre-diabetic and diabetic). Islet-resident macrophages from NOD and age- matched C57BL/6 mice were phenotyped by flow cytometry to characterize the expression of SPM receptors (FPR2, GPR18, BLT1, ChemR23 and LGR6).

Results: The metabolomes of arachidonic, eicosapentaenoic and docosahexaenoic acid were found altered in T1D patients. Mostly significant increased levels of pro-inflammatory lipids (TXB_2, PGE-2, 5,12 and 15-HETE) but also of anti-inflammatory SPMs (LXA_4, Mar1 and 2) and their precursors (14 and 17-HDOHE) were obtained. Similarly, results found in mice showed an altered circulating signature in diabetic versus pre-diabetic animals but no differences between young and pre-diabetic groups. Moreover, in pancreatic islet-macrophages, a higher expression of SPM receptors was found in NOD vs C57BL/6 strain (especially for GPR18, BLT1 and ChemR23).

Conclusions: These findings support the use of lipid mediator signatures as T1D biomarkers and identify some axis present in islet-macrophages (i.e. Resolvin E1-2 – chemR23 or Resolvin D2 – GPR18) that can be explored as therapeutic targets.

POSTER 77

CHARACTERIZATION OF HUMAN MACROPHAGES BY LC-MS BASED ANALYSIS OF OXYLIPINS AND PROTEIN ABUNDANCE FROM A SINGLE CELL PELLET

REBECCA PFAFF, Nicole M. Hartung, Malwina Mainka, Lilli Zinnert and Nils Helge Schebb

Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Gaussstr. 20, 42119 Wuppertal, Germany

(Chronic) Inflammation is hallmark of many diseases. In order to deal with this rising issue, it is crucial to better understand the regulation of inflammation and find new approaches suppressing severity of inflammatory processes. Macrophages play central role in inflammation and host defense producing important downstream signaling molecules. Of these molecules, oxylipins are of particular interest which are important lipid mediators formed via the arachidonic acid (ARA) cascade.

In order to better understand the regulation of the ARA cascade in human macrophages we developed a quantitative multi-omics approach. Combining liquid-chromatography mass spectrometry based targeted oxylipin metabolomics and targeted proteomics enables quantitative analysis of the oxylipin levels as well as the protein abundance from a single cell pellet. This method allows us to quantify all human cyclooxygenases (COX-1 and -2) and relevant lipoxygenase (LOX) pathway enzymes (5-, 12, 15-LOX, 15-LOX-2 and FLAP) as well as more than 200 oxylipins in parallel.
Primary human macrophages derived from blood monocytes were differentiated into M0-, M1- and M2-like using colony-stimulating factor (CSF)-1 (M2-like) or CSF-2 (M1-like) for 8 days and additional IL-4 (M2-like) or IFNgamma (M1-like) for the last 48 h. The different types of polarization led to specific protein patterns and corresponding oxylipins: M0-like macrophages show only low amounts of 12-LOX and COX-1 which probably originate from platelet impurities. In addition to COX-1, 5-LOX and FLAP were also present in M1-like macrophages leading to relevant formation of COX- and 5-LOX-products. Only M2-like macrophages showed 15-LOX and 15-LOX-2 abundance and overall higher oxylipin levels compared to other types. Stimulation with bacterial lipopolysaccharide led to a remarkable induction of COX-2 abundance and products whereas LOX pathways were not affected.

On poster we show a successful application of a newly developed multi-omics approach on the investigation of the ARA cascade in immune cells. From a single cell pellet, the formation of oxylipins can be directly correlated to the abundance of the enzymes of the ARA cascade. This method will help us to gain a more comprehensive understanding of the regulation of inflammatory processes and to investigate its modulation by novel anti-inflammatory drugs and phytochemicals.

POSTER 78

DEVELOPMENT OF A CHIRAL SFC-MS/MS AND REVERSED-PHASE LC-MS/MS PLATFORM FOR THE QUANTITATIVE METABOLIC PROFILING OF OCTADECANOID OXYLIPINS

ALESSANDRO QUARANTA[1], Benedikt Zöhrer[2,3], Johanna Revol-Cavalier[1,4], Kurt Benkestock[5], Laurence Balas[6], Camille Oger[6], Gregory S. Keyes[7], Åsa M. Wheelock[2,3], Thierry Durand[6], Jean-Marie Galano[6], Christopher E. Ramsden[7], Mats Hamberg[1,4], Craig E. Wheelock[1,2]

1) Unit of Integrative Metabolomics, Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden
2) Department of Respiratory Medicine and Allergy, Karolinska University Hospital, 171 76, Stockholm, Sweden
3) Respiratory Medicine Unit, K2 Department of Medicine Solna and Center for Molecular Medicine, Karolinska Institutet, 171 76, Stockholm, Sweden
4) Larodan Research Laboratory, Karolinska Institutet, 171 65, Stockholm, Sweden
5) Waters Sweden AB, 171 65, Stockholm, Sweden
6) IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France
7) Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

Oxylipins consist of a broad group of lipids that are formed from fatty acids by pathways involving at least one step of dioxygen-dependent oxidation. The most well-studied class of oxylipins are the eicosanoids derived from 20-carbon fatty acids. Octadecanoids are broadly defined as oxylipins derived from 18-carbon fatty acids both by enzymatic and radical-mediated oxidation. Even though 18-carbon fatty acids are the primary dietary lipid source, the role of octadecanoids has commonly been overlooked in human physiology. Recent investigations have shown their involvement in multiple disease processes including pain modulation and thermogenesis, as well as regulation of inflammation and immune function. A major impediment to the study of octadecanoids is a lack of dedicated analytical methods. Enzymatic biosynthesis of octadecanoids results in the enzyme-dependent stereospecific production of compounds, whereas synthesis by autoxidation produces racemic mixtures. We therefore developed an integrated workflow combining chiral separation by supercritical fluid chromatography (SFC) and reversed-phase liquid chromatography (LC) coupled to tandem-MS detection for quantification of a broad panel of octadecanoids. The platform includes 70 custom-synthesized analytical and internal standards to extend the coverage of the octadecanoid synthetic pathways. A total of 103 octadecanoids could be separated by chiral SFC and complex enantioseparations could be performed in <13 minutes, while the achiral LC method separated 67 species in 13.5 minutes. The LC method provided a robust complementary approach with greater sensitivity relative to the SFC method. Both methods were validated in solvent and surrogate matrix in terms of linearity, lower limits of quantification (LLOQ), recovery, accuracy, precision, and matrix effects. Instrumental linearity was good for both methods ($R^2>0.995$) and LLOQ ranged from 0.03-6.00 ng/mL for SFC and 0.01-1.25 ng/mL for LC. The average accuracy in solvent and surrogate matrix ranged from 89-109% in SFC and from 106-220% in LC, whereas coefficients of variation (CV) were <14% (at medium and high concentration) and 26% (at low concentration). Validation in surrogate matrix showed negligible matrix effects (<16% for all analytes) and average recoveries ranged from 71-83%. The combined methods provide a platform to investigate the biological activity of octadecanoids and expand our understanding of these little-studied compounds.

POSTER 79

RESOLVIN D1 AND D2 REDUCE INFLAMMATORY RESPONSE TO SARS-COV-2

ANTONIO RECCHIUTI, Domenico Mattoscio, Sara Patruno, Giulia Ferri, Matteo Mucci, Romina Pecce, Antonella Pomilio, Mario Romano

Università “G. d'Annunzio”, University of Chieti Department of Medical, Oral, and Biotechnology Science, 66100 Chieti, Italy

The COVID-19 pandemic has made sparkly clear that resolution of inflammation is essential avoid further damage due to an excessive host response and severe symptoms even in fully vaccinated people. This is mostly important in people with cystic fibrosis (CF) who have lung disease, chronic inflammation, and persistent respiratory infection. Given the important pro-resolutive functions of resolvin (Rv) D1 and D2, here we determined their bioactions on CF and non-CF macrophages (MF) following SARS-CoV-2 stimulus.

In CF and non-CF MF stimulated (3 h) with the SARS-CoV-2 spike protein 1 (S1), RvD1 and RvD2 (each 10 nM) significantly stopped the release of chemokines interleukin (IL)-8, monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein (MIP)1 beta that are essential for driving further leukocyte recruitment during SARS-CoV-2 infection. RvD1 and RvD2 also blunted tumor necrosis factor (TNF) alpha and IL-6 secretion that were increased by S1 selectively in non CF MF. These counterbalancing actions of RvD1 and RvD2 were also evident following a therapeutic administration of RvD1 and RvD2 to MF after stimulation with S1.

Mechanistically, we found that RvD1 and RvD2 both restored the expression of miR-16 and miR-29a that were downregulated by S1 in CF and non-CF MF and stop NF-kB signaling and MF hyperactivation.

RvD1 and RvD2 also significantly activated bacterial clearing phagocytosis of CF and non-CF MF during *P. aeruginosa* and S1 stimulation.

Together, these findings provide the first evidence regulatory activities of resolvins on inflammation and anti-microbial responses of MΦ to SARS-CoV-2, highlighting the essential roles of SPM in the regulating human immunity against this virus.

POSTER 80

PCOS: A CHRONIC DISEASE THAT FAILS TO PRODUCE ADEQUATELY SPECIALIZED PRO-RESOLVING LIPID MEDIATORS (SPMS).

PEDRO-ANTONIO REGIDOR[1,7]*, Xavier de la Rosa[2], Anna Müller[3], Manuela Sailer[4], Fernando Gonzalez Santos[5], Rafael Gracia Banzo[6] and Jose Miguel Rizo[7]

1) Exeltis Healthcare. Adalperostr. 84, 85737 Ismaning, Germany

2) Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston. 60 Fenwood Road Boston, MA 02115; USA; xdelarosa@bwh.harvard.edu.

3) Exeltis Healthcare. Adalperostr. 84, 85737 Ismaning, Germany; anna.mueller@exeltis.com.

4) Exeltis Healthcare. Adalperostr. 84, 85737 Ismaning, Germany; manuela.sailer@exeltis.com.

5) Solutex SA. Avenida de la Transición Espanola 24, 28108 Alcobendas. Spain; fgsantos@solutexcorp.com.

6) Solutex GC SL. Parque Empresarial Utebo, Avda. Miguel Servet nº 81, 50180 Utebo, Spain; rafael.gracia@insercolab.com.

7) OTC Chemo. Manuel Pombo Angulo 28- 4th floor, 28050 Madrid. Spain; josemiguel.rizo@chemogroup.com

Introduction: Polycystic Ovary Syndrome (PCOS) is an endocrinologic disorder that affects 5-15 % of women of their reproductive age and is a frequent cause of infertility. Major symptoms include hyperandrogenism, ovulatory dysfunction, and often obesity and/or insulin resistance. PCOS also represents a state of chronic low-grade inflammation that is closely interlinked with the metabolic features. "Classical" pro-inflammatory lipid mediators like prostaglandins (PG), leukotrienes (LT), or thromboxanes (TX) are derived from arachidonic acid (AA) and are crucial for the initial response. Resolution processes are driven by four families of so-called specialized pro-resolving mediators (SPMs): resolvins, maresins, lipoxins, and protectins. The study aimed to establish lipid mediator profiles of PCOS patients compared to healthy women to identify differences in their resolutive and pro-inflammatory lipid parameters.

Material and Methods: Fifteen female patients (18-45 years) were diagnosed with PCOS according to Rotterdam criteria, and five healthy women, as comparator group, were recruited for the study. The main outcome measures were: Pro-inflammatory lipid mediators (PG, LT, TX) and their precursor AA; SPMs (Resolvins, Maresins, Protectins, Lipoxins), their precursors EPA, DHA, DPA, and their active biosynthesis pathway intermediates (18-HEPE, 17- HDHA, 14-HDHA).

Results: The level of pro-inflammatory parameters in serum was significantly higher in PCOS-affected women. The ratio [(sum of pro-inflammatory molecules) / (sum of SPMs plus hydroxylated intermediates)] reflecting the inflammatory state was significantly lower in the group of healthy women.

Conclusion: There is a strong pro-inflammatory state in PCOS patients. Further research will clarify whether supplementation with SPMs or their precursors may improve this state.

POSTER 81

FUNCTIONAL CHARACTERIZATION OF KNOCK-IN MICE EXPRESSING HUMANIZED ALOX15 AND ALOX15B MUTANTS IN MOUSE INFLAMMATION MODELS.

FLORIAN REISCH, Marjann SchÄfer, Dagmar Heydeck, Dominika Labuz and Hartmut Kuhn

Department of Biochemistry, Charité - University Medicine Berlin, Charitéplatz 1, 10117 Berlin, Germany

Mammalian arachidonic acid lipoxygenases (ALOXs) have been implicated in the pathogenesis of inflammatory diseases and pro- as well as anti-inflammatory effects have been reported for different ALOX-isoforms. Human ALOX15 and human ALOX15B oxygenate arachidonic acid (AA) to 15-hydroperoxy arachidonic acid (15S-HpETE), whereas 12S- and 8S-HpETE are dominantly formed by their mouse orthologs. This functional difference impacts the biosynthetic capacity of the enzymes for pro- and anti-inflammatory eicosanoids. To explore the functional consequences of humanization of the reaction specificity of mouse Alox15 and Alox15b in vivo, we developed Alox15 (Leu353Phe) and Alox15B (Tyr603Asp+His604Val) knock-in mice (KI) expressing AA 15-lipoxygenating proteins instead of the AA 12- or 8-lipoxygenating wildtype enzymes and characterized these mice with respect to their basic functional properties. Alox15-KI and Alox15b-KI mice are viable and reproduced normally. Male Alox15b-KI mice gained significantly less body weight than outbred wildtype controls and this premature growth arrest may be related to a defective hematopoietic system of the genetically modified animals. Such developmental retardations were not observed for Alox15-KI mice.

Next, we tested the two genetically modified mouse strains in two different mouse inflammation models. In the dextran sodium sulfate induced experimental colitis model female Alox15-KI mice lost significantly less bodyweight during the acute phase of inflammation and recovered faster during the resolution phase when compared with outbred wildtype controls. On the other hand, female Alox15b-KI mice lost significantly more bodyweight during the acute phase of inflammation and recovered less rapidly during resolution. These data suggested that humanization of the reaction specificity of Alox15 protected mice from experimental colitis whereas the humanization of Alox15b sensitized the animals in this colitis model.

When we tested the two genetically modified mouse strains in the Freund's complete adjuvant induced paw edema model humanization of the reaction specificity of Alox15b protected the genetically modified mice from inflammation when the paw volume was used as readout parameter. In contrast, pressure (von Frey-test) and heat (Hargraeves-test) induced pain sensations were not significantly different when genetically modified mice and outbred wildtype controls were compared. For Alox15-KI mice we did not observe significant differences in either of these model systems.

POSTER 82

PROSTAGLANDIN E2 EXERTS BIPHASIC DOSE RESPONSE ON THE PREBÖTZINGER COMPLEX RESPIRATORY-RELATED RHYTHM

Jan Philipp Reising, Wiktor S. Phillips, Naify Ramadan, ERIC HERLENIUS

Center for Molecular Medicine, L8:01, Solna, Sweden

Inflammation in newborns can disturb respiration and lead to life-threatening apneas. One of the inflammatory mediators involved in this process is Prostaglandin E_2 (PGE_2). Here we investigate how PGE_2 modulates the generation of the inspiratory rhythm in the preBötzinger complex (preBötC). Rhythmic medullary slice preparations were exposed to increasing concentrations of PGE_2 (1nM-1µM) and changes in motor output recorded. While low concentrations of (1-10nM) PGE_2 slowed the inspiratory rhythm, a higher concentration (1µM) sped it up. Probing the prostanoid receptors (EP1-4R) with specific pharmacology revealed that both EP2R and EP3R are involved in PGE_2's effect on the inspiration rhythm. Selective activation of EP3R is sufficient to lengthen the burst period, while activation of EP2R shortens it. However, simultaneous activation of both is necessary for the biphasic response to PGE_2 at low and high concentration. Single-cell RNA-Seq data revealed that EP2R transcripts (Ptger2) are differentially expressed in putatively excitatory neurons of the preBötC, while EP3R transcripts (Ptger3) are preferentially found in mixed inhibitory neurons. Astrocytes in the brainstem respiratory centers are known to release PGE_2 in response to stress. Using dual calcium imaging of astrocytes and neurons in the preBötC we investigate the effect of low concentrations of PGE_2 on calcium signaling. In summary, the inspiratory rhythm generator has a biphasic response to increasing concentrations of PGE_2, which is mediated by EP2R and EP3R.

POSTER 83

EFFECTS OF ANDROGENS ON FAHFA CONCENTRATIONS IN PERIGONADAL ADIPOSE TISSUE THROUGH ADTRP GENE ACTIVITY

MARTIN RIECAN, Jan Silhavy, Tomas Cajka, Radka Trubacova and Ondrej Kuda

Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

Fatty acid esters of hydroxy fatty acids (FAHFAs) are a recently discovered class of lipids. This discovery of FAHFAs opened a new horizon in bioactive lipid research. Although little is yet known about the biosynthesis of these lipids, it has been established that they are produced endogenously in the great number of combinations of the two acyl chains linked by estolide bond. Triacylglycerols in adipose tissue are considered as a reservoir for these lipid molecules derived from adipose tissue i.e. lipokines that act as hormonal regulators and coordinate an array of cellular processes. Among those biological activities described to date, FAHFAs have anti-diabetic and anti-inflammatory effects and they protect against colitis by regulating gut innate and adaptive immune responses. Also, recently discovered is the androgen-dependent tissue factor pathway inhibitor regulating protein (ADTRP), which is likely to play an important role in diseases associated with bleeding and blood clotting. Later research revealed no less interesting ability of ADTRP to hydrolyze FAHFA at the estolide binding site. Our work focuses on the direct link between two separately reported mechanisms for regulating ADTRP gene expression by androgens and the ability of ADTRP to affect FAHFA levels in the organism. In the results, we can see significantly higher concentrations of FAHFA levels in wild-type female mice compared to male mice in perigonadal adipose tissue. Later in our experiments, we tried to replicate the same concentration pattern by depletion of androgens using castration surgery of 9 weeks old male rats in the group of FAHFA analytes. Measured data are supported by lipidomics analysis and quantitative determination of the ADTRP gene by qPCR.

Supported by Czech Academy of Sciences (Lumina quaeruntur LQ200111901)

POSTER 84

EVALUATION OF SPECIALISED PRO-RESOLVING MEDIATORS AND THEIR RECEPTORS IN OSTEOCLAST LINEAGE CELLS FROM HEALTH AND DISEASES

PATRICIA RIEDLOVA, Cecilia Ansalone, Kieran Woolcock, Shatakshi Sood, Carl S Goodyear

Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow G12 8TA, UK

Abstract: Osteoclasts (OCs) are multinucleated bone-resorbing cells that work in concert with bone-forming osteoblasts to maintain physiological bone remodelling. In certain diseases (i.e., rheumatoid arthritis (RA)), the balance is tipped in favour of OCs, resulting in uncontrolled bone destruction. Specialised pro-resolving mediators (SPMs) are lipids biosynthesised from essential fatty acids. They play a crucial role in resolution of inflammation and recent studies suggest a role in bone remodelling. Notably, resolvin E1 (RvE1) inhibits osteoclastogenesis and
bone resorption in an inflammatory murine model (1) and RAW264.7 cell lines (2). However, the capacity of RvE1 to inhibit primary human
pre-cursors into mature osteoclasts is not known. Moreover, the expression
of SPM receptors known to bind RvE1 (e.g., CMKLR1, ALX4) on this lineage is not well understood.
In this study, we focussed on the expression of known SPM receptors in human OCs and OC pre-cursors. Initial analysis used RNAseq data of healthy monocytes versus monocytes from patients with different autoimmune diseases, as well as healthy osteoclasts. Protein expression in OC cultures was confirmed via immunofluorescence staining. We further evaluated the effect of RvE1 on OC differentiation and function in the presence of the receptor activator of nuclear factor-kappa B ligand
(RANK-L) ± tumour necrosis factor (TNF), where TNF was added to mimic the inflammatory environment.
The results suggest that SPM receptors are differentially expressed in monocytes isolated from healthy donors compared to those from a range of inflammatory diseases, including RA, systemic lupus erythematosus, and systemic sclerosis. Furthermore, expression of SPM receptors changed between monocytes and mature OCs. Further validation via imaging also revealed differences in CMKLR1 and ALX4 expression in OCs and OC pre-cursors. Finally, exposure to RvE1 via these receptors reduced OC formation and bone resorption in the presence of RANK-L and TNF, but not with RANK-L alone.
In conclusion, our study demonstrates a specific expression pattern of SPM receptors in diseases and confirms the role of RvE1 in OC differentiation and function. These data highlight the importance of investigating the effects of SPMs in disease and provide rationale for further research into their potential role as a novel treatment modality.

(1) El Kholy, K., Freire, M., Chen, T., & Van Dyke, T. (2018). Resolvin E1 Promotes Bone Preservation Under Inflammatory Conditions.
Frontiers In Immunology, 9. doi: 10.3389/fimmu.2018.01300
(2) Funaki, Y., Hasegawa, Y., Okazaki, R., Yamasaki, A., Sueda, Y., & Yamamoto, A. et al. (2018). Resolvin E1 Inhibits Osteoclastogenesis and Bone Resorption by Suppressing IL-17-induced RANKL Expression in Osteoblasts and RANKL-induced Osteoclast Differentiation. Yonago Acta Medica, 61(1), 008-018. doi: 10.33160/yam.2018.03.002

POSTER 85

EICOSANOID METABOLISM IN SELECTED REPRESENTATIVES OF BONY FISH WITH PARTICULAR EMPHASIS ON THE ALOX15/ALOX15B LIPOXYGENASE PATHWAY

SOPHIE ROIGAS, Dagmar Heydeck and Hartmut Kuhn

Department of Biochemistry, Charité - University Medicine Berlin, Charitéplatz 1, 10117 Berlin, Germany

Eicosanoids form a class of lipid-mediators that have been implicated in physiological processes but also in the pathogenesis of inflammatory, hyperproliferative and neurological diseases. Eicosanoids are biosynthesized via three different pathways (COX-pathway, LOX-pathway, Cyt-P450-pathway). Eicosanoid synthesizing enzymes are widely distributed in highly developed plants and animals. In lower plants, in protozoa and in metazoa they occur less frequently and in the genomes of currently sequenced procaryotes corresponding genes are rare. A systematic search for lipoxygenase genes in the genomes of currently sequenced bacteria suggested that less then 0.5 % of all Bacteria carry ALOX genes. No ALOX genes have been identified in Archaea. In mammals, ALOX genes occur more frequently and ALOX15 orthologs have been described in more than 160 metatherian and eutherian species. Interestingly, no ALOX15 genes have been detected in Prototheria but genes encoding for other ALOX-isoforms (ALOX5, ALOX15B, ALOXE3) were present in this class of ancient mammals.

Fish represent a major clade of vertebrates and almost two thirds of all terrestrial vertebrates are classified as fish. We screened the genomic databases for ALOX15 sequences in bony fish and only obtained 7 hits. Some of these sequences were incomplete or lacked the non-heme iron ligands. For expression and functional characterization, we selected the sequences of *Nothobranchius furzeri* (XP_015813570.1), *Pundamilia nyererei* (XP_005753048.1) and *Scleropages formosus* (XP_018588735.1). The coding sequences of these genes were cloned into pro- and/or eukaryotic expression vectors, the enzymes were expressed as N-terminal his-tag fusion proteins in *E. coli* and/or in Sf9 insect cells and the recombinant enzymes were characterized with respect to their protein-chemical and enzymatic properties. We found that neither of these enzymes followed the Triad Concept of reaction specificity. Moreover, they violated the Ala-vs-Gly hypothesis and similar observations have previously been made for zebrafish LOX1. Dual amino acid alignments with all human and mouse ALOX isoforms revealed a similar degree of amino acid conservation (40-50%), which made it impossible to assign these enzymes to any of the human isoforms. Since all characterized fish ALOX-isoforms lack a sizeable membrane oxygenase activity they may not constitute ALOX15 orthologs despite the faulty database annotations.

POSTER 86

METABOLIC REGULATION BY PROSTAGLANDIN E2 IMPAIRS LUNG GROUP 2 INNATE LYMPHOID CELL RESPONSES

Calum Robb, You Zhou, Jennifer Felton, Birong Zhang, Marie Goepp, Privjyot Jheeta, Danielle Smyth, Richard Breyer, Shuh Narumiya, Henry McSorley, Rick Maizels, Jürgen Schwarze, Adriano Rossi, CHENGCAN YAO

The University of Edinburgh Centre for Inflammation Research, Edinburgh UK

Background: Group 2 innate lymphoid cells (ILC2s) play a critical role in asthma pathogenesis. Non-steroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (NERD) is associated with reduced signaling via EP2, a receptor for prostaglandin E_2 (PGE_2). However, the respective roles for the PGE_2 receptors EP2 and EP4 (both share same downstream signaling) in the regulation of lung ILC2 responses has yet been deciphered.

Methods: The roles of PGE_2 receptors EP2 and EP4 on ILC2-mediated lung inflammation were investigated using genetically modified mouse lines and pharmacological approaches in IL-33- induced lung allergy models. The effects of PGE_2 receptors and downstream signals on ILC2 metabolic activation and effector function were examined using in vitro cell cultures.

Results: Deficiency of EP2 rather than EP4 augments IL-33-induced lung ILC2 responses and eosinophilic inflammation in vivo. In contrast, exogenous agonism of both EP2 and EP4 markedly restricts IL-33-induced lung ILC2 responses and eosinophilic inflammation. Mechanistically, PGE_2 directly suppresses IL-33-dependent ILC2 activation through the EP2/EP4-cAMP pathway, which downregulates STAT5 and MYC pathway gene expression and ILC2 energy metabolism. Blocking glycolysis diminishes IL-33-dependent ILC2 responses in mice lacking endogenous PG synthesis but not in PG-competent mice.

Conclusion: We have defined a mechanism for optimal suppression of lung ILC2 responses by endogenous PGE_2-EP2 signaling which underpins the clinical findings of defective EP2 signaling in patients with NERD. Our findings also indicate that exogenously targeting the PGE2-EP2/EP4-cAMP and energy metabolic pathways may provide novel opportunities for treating ILC2-initiated lung inflammation in asthma and NERD.

POSTER 87

IDENTIFICATION AND IN VIVO DETECTION OF 4B-HYDROXYCHOLESTEROL METABOLITES

MARTIN ROUMAIN, Owein Guillemot-Legris, Giulio G. Muccioli

Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, 72, Av. E. Mounier, B1.72.01, 1200 Brussels, Belgium

Oxysterols are oxidized derivatives of cholesterol that are formed by enzymatic processes or through the action of reactive oxygen species. 4b-hydroxycholesterol, an LXR agonist, is the most abundant circulating oxysterol in mice. It is synthesized by the CYP3A4 and CYP3A5, in humans, or their murine orthologues Cyp3a11 and Cyp3a13.
4β-hydroxycholesterol levels are decreased in inflammatory pathologies such as Crohn's disease and obesity. Surprisingly, apart from its long half-life of several days, little is known about its catabolic pathway.

27-hydroxycholesterol is an oxysterol formed by CYP27A1. It is also implicated in inflammatory processes, mainly through its action on LXR, ERalpha and RORgamma. As 4β-hydroxycholesterol, its levels were decreased in patients suffering from inflammatory bowel diseases (IBD), which was directly correlated with the decreased expression of CYP27A1, CYP3A4 and CYP3A5.

In this context, we decided to investigate the catabolism of 4b-hydroxycholesterol, and more specifically its 27-hydroxylation by Cyp27a1.

First, we set up an in vitro Cyp27a1-activity assay to measure the 27-hydroxylation of 4β-hydroxycholesterol. Mitochondria-enriched fractions were isolated from mouse liver and the protocol was validated using cholesterol as a substrate. Then, the protocol was applied to the 27-hydroxylation of 4β-hydroxycholesterol. Using an HPLC-MS Orbitrap with high mass resolution, we observed four peaks corresponding to hypothetical 4β-hydroxycholesterol metabolites. Their levels were decreased by Cyp27a1 inhibitors. In order to identify these metabolites of 4β-hydroxycholesterol, we incubated different oxysterols with CYP3A4, the enzyme responsible for the 4b-hydroxylation of cholesterol. We selected three oxysterols, based on their retention times: 27-hydroxycholesterol, 24(S)-hydroxycholesterol, 25-hydroxycholesterol. The results support the identification of 4β,27-dihydroxycholesterol, 4β,24(S)-dihydroxycholesterol and 4β,25-dihydroxycholesterol.

Finally, we observed an increased signal of putative 4β,27-dihydroxycholesterol in mouse liver and plasma following intraperitoneal administration of 4β-hydroxycholesterol.

POSTER 88

METHOD DEVELOPMENT FOR THE SIMULTANEOUS QUANTIFICATION OF MULTIPLE BIOACTIVE LIPID CLASSES

STEFANIE RUBENZUCKER, Robert Ahrends

University of Vienna, Währinger Strasse 38, 1090, Vienna, Austria

Abstract: Bioactive lipids govern many cellular processes, including cell growth, death, and migration, and are key players in inflammatory processes. [1-4] While approaches have been made to develop an assay for a few of these lipid classes [5], there is yet no comprehensive workflow that can measure all relevant lipid classes in one analytical run. Challenges include a high dynamic range and, most of all, the very different structures and chemical properties of these analytes. [1-5]

We selected analytes from more than 15 different lipid classes to establish our method, including eicosanoids, fatty acids and conjugates, fatty amides, sphingoid bases, ceramides, glycerophosphotidylserines, glycerophosphotidylinositols, and phosphatidic acids. In the first step, we optimized the collision energy and chose adequate transitions for all analytes on a QTrap 6500+. We then tested different solvent compositions and additives with a standard C18 column. Solvents with formic or acetic acid proved to be the best choice for ionization efficiency and chromatographic separation. However, lysolipids could not be separated using this setup, as they showed very poor peak shape and high carryover.

This problem was overcome by adding 5 mM ammonium formate or ammonium acetate - however, this negatively influenced signal intensity and peak shape of many lipid mediators and specific sphingoid bases. Thus, we then tested columns with similar column dimensions but different column chemistry and particle size. Finally, we selected the column that achieved the best performance (signal intensity, chromatographic separation, and peak shape) for all analytes with only formic or acetic acid as solvent additives. Therefore, we report the first LC-MS method that enables the simultaneous measurement of more than 15 different bioactive lipid classes in one LC-MS run.

Literature:

[1] Peng, B., Geue, S., Coman, C., et al., Blood. 2018, 132(5), e1-e12.
[2] Moolenaar, W.H., Ann. N. Y. Acad. Sci. 2000, 905, 1-10.
[3] ChiurchiÃ¹, V., Leuti, A., Maccarrone, M., Front. Immunol. 2018, 9:38.
[4] Obinata, H., Hla, T., Int Immunol. 2019, 31(9), 617-625
[5] Schoeman, J. C., Harms, A. C., van Weeghel, M., Berger, R., Vreeken, R. J., Hankemeier, T., Anal Bioanal Chem. 2018, 410(10), 2551-2568.

POSTER 89

CYSTEINYL-MARESIN 3 INHIBITS IL-13 INDUCED AIRWAY HYPERREACTIVITY THROUGH ALTERNATIVE ACTIVATION OF THE CYSLT1 RECEPTOR

JESPER SÄFHOLM, Willem Abma, Craig E. Wheelock, Sven-Erik Dahlén, and Mikael Adner

Karolinska Institutet, Biomedicum, Q5B, Solnavägen 9, 171 77 Stockholm

Introduction: Maresin-conjugates in tissue regeneration (MCTRs) are recently discovered lipid mediators proposed to reduce inflammation. The aim was to investigate the influence of MCTR3 on interleukin (IL)-13- induced airway hyperreactivity.

Methods: Isolated human small bronchi and tracheas from wild type and genetically modified mice were during culture procedures exposed to IL-13 during 2-4 days in the presence or absence of MCTRs. After culture, responsiveness to contractile agonists were assessed using myographs.

Results: IL-13 treatment increased contractions (Emax) to histamine (His; 143±5% vs. 113±6% for control, $p<0.05$) leukotriene D4 (LTD4; 155±12% vs. 88±8% for control, $p<0.05$) and carbachol (CCh; 137±6% vs. 93±5% for control, $p<0.05$) in human small bronchi, and to 5-hydroxytryptamine (5-HT; 59±4% vs. 25±2% for control, p<0.05) in mouse trachea. Co-incubation of the explanted tissues with MCTR3 reduced the IL-13 induced enhancement of contractions in human bronchi (His: 123±7%, LTD4: 117±9% and CCh: 115±8%, $p<0.05$) and in mouse trachea (5-HT: 23±6%, $p<0.05$). In mouse trachea, this inhibitory effect of MCTR3 was blocked by three different CysLT1 antagonists during IL-13 exposure ($p<0.05$). Likewise, MCTR3 failed to reduce the IL-13-induced 5-HT responsiveness in mice deficient of the CysLT1 receptor. In contrast, co-incubation with LTD4 neither altered the IL-13-induced 5-HT responsiveness, nor the MCTR3-induced reduction of contraction.

Conclusion: Co-culture of IL-13 and MCTR3, but not LTD4, decreased airway hyperreactivity by activation of the CysLT1 receptor. The distinct actions of the two lipid mediators on the CysLT1 receptor suggest an alternative signalling pathway appearing under IL-13 induced inflammatory conditions.

POSTER 90

TARGETED METABOLOMICS SIGNATURES OF CIRCULATING INTERLEUKIN 6 (IL-6): A DISCOVERY AND REPLICATION APPROACH

SAMIRA SALIHOVIC[1], Lars Lind[2]

1) School of Medical Science, Örebro University, Sweden. samira.salihovic@oru.se
2) Department of Medical Science, Uppsala University, Sweden, lars.lind@medsci.uu.se

BACKGROUND: Interlukin-6 is a proinflammatory cytokine involved in many physiological and pathological processes acting via multiple intracellular signaling pathways. Recent investigations have shown that cytokines such as IL-6 possess important abilities that influence glucose and lipid metabolism; however, the underlying mechanisms remain not completely understood. The main aim of this study was to assess associations of IL-6 and metabolism measured by targeted 1H-NMR metabolomics in three independent cohorts of adult individuals.

METHODS: In three population-based cohorts of adult individuals from Sweden: the Epidemiology for Health cohort [PIVUS, n = 2353, median age 60 years], the Prospective Investigation of the Vasculature in Uppsala Seniors [PIVUS, n = 590, median age 80 years]; and the Prospective Investigation of Obesity and Energy Metabolism [POEM, n = 501, median age 50 years] plasma IL-6 concentrations were determined using routine clinical chemistry. Targeted metabolomics was performed using proton nuclear magnetic resonance spectroscopy (1H-NMR). Associations of IL-6 and targeted metabolomics signatures was assessed using a discovery-validation approach with multivariable linear regression.

RESULTS: In the discovery cohort EpiHealth163 non-targeted metabolomic features out of 220 could be associated with IL-6 concentrations following adjustment for age, sex, and correction for multiple testing. 83 associations could then be replicated in PIVUS and POEM. Notably, most of these relationships were negative. The top metabolism markers being replicated in were glycoprotein acetyls, phenylalanine, and several lipid related metabolites.

CONCLUSIONS: Using a targeted 1H-NMR metabolomics discovery-validation approach, we confirmed some of the previously reported association between IL-6 and lipid related metabolites and identified and replicated novel associations. Our results encourage additional studies investigating the underlying mechanisms of our findings.

POSTER 91

SPATIAL ORGANIZATION OF IMMUNE CELLS DURING INFLAMMATION AND EFFECTS OF ANTIINFLAMMATORY AGENTS

TIM J. SCHÄUFELE; Anja Kolbinger; Prof Dr. Dr. Klaus Scholich

Theodor-Stern-Kai 7 60590 Frankfurt am Main Haus 74/75 Germany

An inflammation can be roughly divided in two phases, the proinflammatory phase and the resolution of inflammation. Importantly, an inflammation is formed by several very different regions, which depend on the distance to the center of the inflammation as well the local tissue characteristics (e.g. immune cells, resident cells, vascularization etc.).

These regions present different microenvironments for the immune cells recruited to the tissue inducing specific answers (i.e. production of interleukin (IL)-4, IL-1b or prostaglandin E_2), tailored for the specific needs of each region. While the regulation of the activity of immune cells is well investigated, their localization during an inflammation and the spatial relationships to each other, which reflect the complex interactions between several immune cell types in vivo, are less well understood. Therefore, we quantitative evaluated the spatial distribution of immune cells and signaling mediators in the time course of a zymosan-induced inflammation.

For characterization of immune cell architecture, we used Multi-Epitope-Ligand-Cartography (MELC) technology, a specialized immunohistological imaging technology that allows us to routinely visualize more than 40 fluorescence tags (i.e. fluorescence-labeled antibodies) on the same tissue slice in an automated process. As a result of this process the marker colocalization in regard to their microenvironment and position in the inflamed area can be quantitatively assessed using bioinformatic approaches. To overcome the huge amount of MELC image data, we established an analysis pipeline, starting with generation of a single cell segmentation mask that defines fluorescence signals as cells, based on nuclear staining and pan-cell marker CD45.

Defined cells undergo dimension reduction, phenotype clustering and neighborhood analysis using histology topography cytometry analysis toolbox (histoCAT).

This analysis enables us to identify, group and assign cell phenotype cluster to their cellular neighborhood and superordinate region. We identified different regions arising during an innate inflammation and characterized the inflammatory architecture focusing on the spatial interactions of immune cells with each other and in regard to their distance to the core region (neighborhood analysis). Further, we observed a change in this architecture by application of the non-steroidal anti-inflammatory drug (NSAID) Meloxicam.

POSTER 92

QUANTITATIVE ANALYSIS OF EICOSANOIDS USING SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC) ON POLYSACCHARIDE COLUMN COUPLED TO SINGLE QUADRUPOLE MASS SPECTROMETRY

LOUIS SCHMIDT, Laura Burmeister, Ulrike Garscha

Pharmaceutical and Medicinal Chemistry Institute of pharmacy University of Greifswald Friedrich-Ludwig-Jahn-Str. 17 17489 Greifswald Germany

Eicosanoids are lipid mediators with pro- and antiinflammatory properties that are generated from arachidonic acid (AA). While prostaglandins (PG) and thromboxane (TX) are formed via cyclooxygenases, lipoxygenases convert AA to leukotrienes, lipoxins and hydroxyeicosatetraenoic acids (HETE). Despite these lipid mediators are known for decades, quantitative determination in biological samples is still challenging due to minute occurrence, instability and existence of regio- and stereoisomers. Additionally, a broad range of polarity requires prolonged run times for chromatographic separation, which is inconvenient in the daily routine, cost intensive and ecologically harmful. The orthogonality of supercritical fluid chromatography (SFC) along with unique properties of supercritical CO2 such as low viscosity and high diffusivity facilitate efficient and enhanced lipid mediator separation in terms of shorter runtimes, less solvent consumption and additional isomer recognition.

Here, we aimed to develop and validate a SFC-MS method for quantification of typical occurring eicosanoids in biological samples without required collision-induced fragmentation. Methanol, a commonly applied modifier and silica-based columns did not provide sufficient separation of regioisomers. Chiral amylose-based columns instead offered improved separation and isomer recognition. Furthermore, modifier combination of 2-propanol and acetonitrile increased resolution while reducing retention time. We revealed a baseline separation of isobaric eicosanoids within 15 min, which enables detection of 11 oxylipins by MS covering a range from 78 ng/mL to 2,5 microg/ml. The method got validated in terms of linearity, LOQ, accuracy, precision and recovery according to EMA guidelines.

Finally, we proved the applicability of the method by quantifying eicosanoid levels in human primary blood cells. In conclusion, we established a SFC-MS method to quantify different eicosanoids with a wide range of polarity while maintaining baseline separation of isobaric analytes without complex technical requirements for multiple reaction monitoring.

POSTER 93

STRUCTURAL AND FUNCTIONAL STUDY ON MEMBRANE-ASSOCIATED PROTEINS IN EICOSANOID AND GLUTATHIONE METABOLISM

RAJKUMAR SINGH and Jesper Z Haeggström

Department of Medical Biochemistry and Biophysics Division of Physiological Chemistry- II, Solnavägen 9, Karolinska Institute, Stockholm, Sweden

The MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) family consists of various membrane integrated proteins which are present in the endoplasmic reticulum (ER) and nuclear membrane (NM)1. These proteins play important roles in human physiology and diseases, such as allergic and chronic inflammation. Thus, these membrane proteins are important targets for development of new anti-inflammatory drugs[1,2]. Some MAPEGs are microsomal glutathione S-transferases (MGSTs), which are important for detoxification, protection against lipid peroxidation and also LTC_4 formation from LTA42. In recent times, some family members have been structurally and functionally characterized[1,2]. MGST2 high resolution protein structure was recently solved in apo and GSH bound conformation by lipidic cubic phaseprotein crystallization method[2].

In this study, we are using both protein crystallization and Cryo EM techniques to determine high resolution structures (in apo and substrate bound conformation) of two human MAPEG proteins, MGST1 and MGST3, along with biochemical characterization. MGST1 and MGST3 are expressed in *Pichia pastoris* and further stable protein has been purified in detergent solution which was used for biochemical analysis and also for protein crystallization. These human membrane proteins are first purified in n-dodecyl-Î²-D-maltopyranoside (DDM) detergent and then analysed for thermal stability enzyme activities. Our initial results indicate that MGST3 protein, but not MGST1, catalyses leukotriene C4 (LTC_4) synthesis via conjugation of glutathione (GSH) and LTA4, a transient epoxide intermediate which is derived from metabolism of arachidonic acid along the 5-lipoxygenase pathway. MGST3 also displays GSH dependent lipid peroxidase activity against hydroperoxyl fatty acids.

1. Thulasingam, M., and Haeggström, J.Z. Integral Membrane Enzymes in Eicosanoid Metabolism: Structures, Mechanisms and Inhibitor Design J Mol Biol 423,18,4999-5022, 2020.

2. Thulasingam, M., Orellana, L., Nji, E., Ahmad, S., Rinaldo-Matthis, A., and Haeggström, J.Z. Crystal structures of human MGST2 reveal synchronized conformational changes regulating catalysis. Nat Commun 12, 1728, 2021.

POSTER 94

HIGH-THROUGHPUT SCREENING OF DRUG LIBRARY COMBINED WITH MPGES-1 INHIBITOR USING MULTICELLULAR TUMOR SPHEROIDS AND INSIGHT INTO THE MECHANISM OF SYNERGY

Brinton Seashore-Ludlow[1]#, AHLEM ZAGHMI[2]#, Julia Steinmetz-Spähs[2], Long Jiang[2], Mingmei Shang[2], Fredrik Wermeling[2], Marina Korotkova[2], Päivi Östling[1], Per-Johan Jakobsson[2], Karin Larsson[2]*

# *Authors contributed equally to the work*

1) Department of Oncology-Pathology, Science for Life Laboratory, Karolinska Institute, Stockholm, Sweden

2) Rheumatology Unit, Department of Medicine, Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

High-throughput drug screening is undoubtedly a valuable resource for detecting novel anti-cancer agents. So far, monolayer cell cultures have been the most prevalent models in drug screening endeavors. However, these models failed to recapitulate the complex microenvironment of solid tumors and displayed low bench-to-bed translational efficiency. In contrast, three-dimensional (3D) cell cultures, such as multicellular tumor spheroids (MCTS), are becoming a crucial tool in cancer research, as they mimic the architecture and microenvironment of in vivo tumors. These models provide a great potential for studying the biological assets of cancers and represent a promising platform for drug discovery. In this work, we aimed to develop neuroblastoma (NB) MCTS for high- content drug screening with an mPGES-1 inhibitor. We also set out to shed the light on the potential mechanism of action of mPGES-1 inhibitor-drug combinations acting highly synergistically. Multiple conventional agents, across a variety of therapeutic categories and with different modes of action, were tested. Following systematic examination of the combination effects of single-drugs and pairwise combinations, GFP-transfected MTCS were used in a secondary screen to better characterize the hits. Finally, real-time kinetic caspase 3/7 apoptosis and efflux pumps inhibition effects were assessed. In summary, this work illustrates how NB MCTS-based high-throughput drug screening yields potential to reveal previously unrecognized mPGES-1 inhibitor-drug combinations. It also highlights the concept of synergy and gives some insights into the mechanism of synergy between an mPGES-1 inhibitor and chemotherapeutics.

POSTER 95

MODIFICATION OF SERUM PHOSPHOLIPID FATTY ACID PROFILES IN PRETERM INFANTS AFTER SUPPLEMENTATION WITH PARENTERAL LIPIDS AND ENTERAL DHA AND AA

Ulrika Sjöbom, Mats X Andersson, Aldina Pivodic, Anna-My Lund, Mireille Vanpee, Ingrid Hansen-Pupp, David Ley, Dirk Wackernagel, Karin SÄvman, Lois EH Smith, Chatarina Löfqvist, Ann Hellström, and ANDERS K NILSSON

Section for Ophthalmology, Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Background: Infants born extremely preterm are at risk of postnatal deficit of long-chain polyunsaturated fatty acids (LCPUFAs) with potential effects on morbidities and neurodevelopment.

Objective: In preterm infants, determine longitudinal fatty acid profiles and modification with enteral supplementation with the LCPUFAs arachidonic (AA) and docosahexaenoic acid (DHA), and parenteral lipids.

Design: Secondary analysis of a randomized control study of infants born <28 weeks of gestation (GA). Infants in the intervention group (n=101) received an enteral single-cell oil supplement containing AA:DHA at 100:50 mg/kg/day; the control group (n=103) received standard care. Serum samples (n=1660) were collected from birth to term equivalent age, phospholipid fatty acids were determined by GC-MS, and levels expressed in relative (mol%) and absolute (µmol-l) units.

Results: Infants in the AA:DHA and control groups were well matched with a mean (SD) GA of 25.5 (1.4) weeks. Infants receiving the lipid enteral supplement reached higher relative levels of AA between postnatal week 7 and 13 (range in mean difference 0.78 to 2.19 mol%) and of DHA between week 8 and 13 (0.26 to 0.46 mol%), and higher absolute levels of AA between week 9 and 11 (48.3 to 59.5 µmol-l). Other quantified fatty acids were unaffected by the supplementation.

Fatty acids profiles of cord blood and day 1 were similar in all individuals, then showed heterogeneity until four weeks of life, then became similar towards the end of the study at 40 weeks postmenstrual age. The high variability in fatty acid profiles during the first weeks coincided with differences in parenteral lipid intake. High parenteral lipid intake was associated with lower serum levels of AA and DHA.

Conclusion: Enteral AA:DHA supplementation increases these fatty acids in infant serum with little effect on other fatty acids. Parenteral delivery of lipids strongly impacts both relative and absolute LCPUFA levels.

POSTER 96

BIOSYNTHESIS OF 15-DEOXY-Δ12,14-PROSTAGLANDIN J2-GLUTATHIONE AND -CYSTEINE CONJUGATES IN MACROPHAGES AND MAST CELLS

Julia Steinmetz-Späh[1], JIANYANG LIU[1], Rajkumar Singh[3], Maria Ekoff[2], Xiao Tang[3], Filip Bergqvist[1], Helena Idborg[1], Pascal Heitel[4], Elin Rönnberg[2], Daniel Merk[4], Jesper Z. Haeggström[3], Gunnar Nilsson[2], Dieter Steinhilber[4], Karin Larsson[1], Marina Korotkova[1], Per-Johan Jakobsson[1]

1) Division of Rheumatology, Department of Medicine, Solna, Karolinska Institutet and Karolinska University Hospital, SE-171 76 Stockholm, Sweden
2) Division of Immunology and Allergy, Department of Medicine, Solna, Karolinska Institutet and Karolinska University Hospital, SE-171 76 Stockholm, Sweden
3) Division of Physiological Chemistry 2, Department of Medical Biochemistry and Biophysics, Biomedicum 9A, Karolinska Institutet SE-171 65 Stockholm, Sweden
4) Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, Max-von-Laue-Straβe 9, 60438, Frankfurt, Germany

15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15dPGJ$_2$), an endogenous ligand of PPAR gamma receptor, is a bioactive metabolite of prostaglandin D_2 (PGD$_2$). It has been shown to have pro-resolving effects in many pre-clinical models. By forming Michael adducts with cysteine residues on targeted proteins, 15dPGJ$_2$ inhibits inflammation through interference/interaction with several signalling pathways like NFκB, Keap1 or Hif1.

Here, we studied the biosynthesis and metabolism of 15dPGJ$_2$ via the conjugation to GSH, forming 15dPGJ$_2$-GS and 15dPGJ$_2$-Cysteine (15dPGJ$_2$-Cys) conjugates in murine (Raw 264.7 and BMDM) and human (primary mast cells) cells under natural stimuli, such as LPS and IgE antibody. Our data also suggested the involvement of microsomal glutathione S-transferase 3 (MGST3) in the formation of 15dPGJ$_2$ conjugates. Moreover, by inhibiting microsomal prostaglandin E synthase-1 (mPGES-1), which catalyses the conversion from PGH$_2$ to pro-inflammatory PGE$_2$, we observed clear shunting into PGD$_2$/15dPGJ$_2$/15dPGJ$_2$-Cys pathway.

Altogether, this study highlights the endogenous formation of 15dPGJ$_2$-GS and 15dPGJ$_2$-Cys in murine and human immune cells, which was preserved and elevated upon mPGES-1 inhibition. The present study encourages further investigations on the potential bioactivities of these novel cyclopentanones, as well as the pro-resolving actions upon mPGES-1 inhibition.

POSTER 97

EFFECTS OF MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 (MPGES-1) INHIBITION ON RESISTANCE ARTERY TONE IN PATIENTS WITH END STAGE KIDNEY DISEASE

JULIA STEINMETZ-SPÃH[1], Samsul Arefin[2], Karin Larsson[1], Jabin Jahan[1], Neja Mudrovcic[2], Lars Wennberg[3], Peter Stenvinkel[2], Marina Korotkova[1], Karolina Kublickiene[2], Per-Johan Jakobsson[1]

1) Division of Rheumatology, Department of Medicine, Solna, Karolinska Institutet & Karolinska University Hospital, Stockholm, Sweden
2) Division of Renal Medicine, Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska University Hospital, Stockholm, Sweden
3) Division of Transplantation Surgery, Department of Clinical Science, Intervention and Technology, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden

A major concern with NSAIDs, selectively inhibiting cyclooxygenase2 (COX-2), is the increased risk for severe cardiovascular events, especially in patients suffering from chronic inflammation. The cardiovascular adverse effects of NSAIDS are widely ascribed to be caused by an imbalance in pro-thrombotic versus anti-thrombotic mediators resulting in thrombotic events, increased arterial blood pressure and heart failure. Inhibition of mPGES-1 the terminal synthase of PGE_2, resembles a novel approach for anti-inflammatory treatment leading besides the reduction of pro-inflammatory PGE_2 to a redirection of excess PGH_2 into the vasodilating PGI_2 pathway. The microvasculature is a central target organ for early manifestations of cardiovascular diseases. Therefore, a better understanding of the prostaglandin system and characterizing the effects of mPGES-1 inhibition in this vascular bed are of interest.

Here we studied the effects of mPGES-1 inhibition on constriction and relaxation of resistance arteries from patients with end stage kidney disease (ESKD) and controls using wire-myography in combination with immunological and mass-spectrometry based analyses. Inhibition of mPGES-1 in arteries from ESKD patients and controls significantly reduced adrenergic vasoconstriction, which was not affected by the COX-2 inhibitors NS-398, Etoricoxib or the COX-1/COX-2 inhibitor Indomethacin. Correspondingly, a significant increase in acetylcholine-induced dilatation and spared PGI_2 levels were observed for mPGES-1 inhibition only. Blockage of IP, EP4 or PPARÎ³ signaling did not restore the reduced constriction following mPGES-1 inhibition. Expression of mPGES-1, COX-1, PGIS and weak expression for COX-2 was found in ESKD and control arteries as well as receptor expression for PGE_2 (EP1-4), thromboxane (TP) and PGI_2 (IP).

In conclusion, our study demonstrates vasoactive effects of mPGES-1 inhibition and suggests that several pathways besides shunting to PGI_2 may be involved in the vasodilating effects following mPGES-1 inhibition in human microvasculature. The presented results motivate for further studies on mPGES-1 inhibition in diseases like Raynaud's phenomenon or myocardial infarction.

POSTER 98

NEW SELECTIVE INHIBITORS OF HUMAN LEUKOTRIENE A4 HYDROLASE

TARVI TEDER, Rajkumar Singh, Jesper Z. Haeggström

Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-17177 Stockholm, Sweden

Leukotriene A4 hydrolase (LTA4H) is a bifunctional zinc-containing enzyme that catalyzes the biosynthesis of a potent pro-inflammatory and immune-modulating lipid mediator, leukotriene B4 (LTB4), and also performs the peptidolytic cleavage of a neutrophil chemoattractant, Pro-Gly-Pro (PGP). Therefore, LTA4H is an important drug target for the resolution of inflammation in different pathophysiological conditions. Elucidation of the crystal structure of LTA4H in a complex with different ligands revealed the binding mode of the LTA_4 substrate and PGP in the L-shaped substrate channel. Based on the structural information, inhibitors can be designed to selectively block only the formation of LTB_4 without affecting the aminopeptidase activity. A lead compound, 4-(4-benzylphenyl)-thiazol-2-amine (ARM1), has been designed and co-crystallized together with PGP analogue in the substrate pocket of LTA4H.
The objective of this work was to synthesize new ARM1-type inhibitors via chalcogen-replacement, and assess their potency and binding properties using different biochemical-biophysical approaches.

Synthesized selective inhibitors, 4-(4-benzylphenyl)-selenazol-2-amine (TTSel) and 4-(4-benzylphenyl)-oxazol-2-amine (TTOx), were used in the hydrolase and aminopeptidase assays of LTA4H. The inhibition of hydrolase activity was assessed based on the biosynthesis of LTB4 by recombinant LTA4H or activated polymorphonuclear neutrophils (PMNs). Both assays indicated that ARM1 is slightly more potent than TTSel and TTOx.
Aminopeptidase assay was carried out with naturally-occurring PGP, and artificial peptides, para-nitroanilide or 7-amido-4-methylcoumarin derivatives. All three inhibitors spared the aminopeptidase activity of LTA4H with PGP, however, the peptidolysis of peptide analogues containing N-terminal hydrophobic amino acids was increased significantly by the inhibitors. Dissociation constants of ARM1, TTSel and TTOx with LTA4H were determined using isothermal titration calorimetry and ThermoFluor assay. The affinity around 300 nM with different inhibitors indicated their similar affinity to LTA4H. Moreover, ThermoFluor assay demonstrated that all selective inhibitors stabilize LTA4H in a similar manner. Finally, the crystal structure of LTA4H in a complex with TTSel or TTOx revealed the similar binding mode and domain movements that were observed with the lead compound, ARM1.

Current results indicate that single chalcogen replacements were not sufficient to improve the potency and binding properties of ARM1, nevertheless, this encourages us to proceed with development and characterization of pharmacologically relevant LTA4H inhibitors.

POSTER 99

THE INFLUENCE OF FADS1 (RS174547) AND FADS2 (RS1535) GENETIC VARIANTS ON BOTH CIRCULATING LEVELS OF LIPID MEDIATORS AND CYTOKINES IN OBESITY.

ESTHER TITOS[1,3,4,7], Cristina López-Vicario[1,3], Mireia Casullerasa[3], Andrea González-Boullin[1], Ferran Aguilar[6], Juan José Lozano[3], José Alcaraz-Quilesa[3], Roger Flores-Costa[1,3], Marta Duran-Güella[3], Ainitze Ibarzábal[2], Juan José Espert[2], Noelia Pérez-Romero[5], and Joan Clària[1,3,4,6,7].

1) Biochemistry and Molecular Genetics Service and 2) Gastrointestinal Surgery Department, Hospital Clínic-IDIBAPS, Barcelona, Spain
3) CIBERehd, Madrid, Spain
4) Molecular Biology CORE, Biomedical Diagnostic Center, Hospital Clínic, Barcelona, Spain
5) Gastroenterology Department, Hospital Universitari Mútua de Terrassa, Terrassa, Spain
6) EF-Clif and Grifols Chair, Barcelona, Spain
7) Department of Biomedical Sciences, University of Barcelona, Barcelona, Spain.

Background and Aim: Obesity is a worldwide health concern of polygenic nature characterized by the presence of an unresolved state of low-grade inflammation and a lipid metabolism imbalance between omega-6 and omega-3 polyunsaturated fatty acids (PUFA). Delta-5 and delta-6 desaturases are enzymatic activities that control the processing and availability of omega-6 and omega-3 PUFA. They are encoded by FADS1 and FADS2, respectively, which are considered candidate genes affecting obesity traits. The aim of the current study was to determine whether previously recognized functional genetic variants rs174547 (FADS1) and rs1535 (FADS2) are associated with obesity, circulating lipid mediators, and cytokines.

Subjects and Methods: Participants were categorized according to body mass index (BMI, kg/m2) as non-obese (BMI < 29.9, n=102) or obese (BMI > 29.9, n=288). DNA samples were genotyped using TaqMan SNP Genotyping assays. Cytokine levels were measured by high-throughput multiplex immunoassay and lipids were analyzed by means of UPLC-MS/MS targeted approach in a representative cohort.

Results: Frequencies of FADS1 and FADS2 genotypes were found to be not different between non-obese and obese individuals under none of the inheritance models analyzed. In both the FADS1 and the FADS2 SNPs, heterozygous carriers of the minor alleles (C and G, respectively) showed decreased levels of a series of pro-inflammatory and vasoactive lipid mediators derived from the omega-6 arachidonic acid. In the rs174547 variant, we found significantly decreased levels of 5-HETE, 8,9-DiHETrE, 11,12-DiHETrE, PGF2a, 14,15-DiHETE and increased ratio of DHA to EPA (3,17 vs 2,03, respectively, p=0.010) in CT individuals compared to TT individuals. In the rs1535 variant AG, individuals showed decreased levels of 8-HETE, 20-HETE, 11,12-DiHETrE, and PGE1, as compared to AA subjects. The association analysis with plasmatic cytokine levels revealed no significant associations with heterozygous carriers of the minor alleles. On the contrary, both minor allele homozygous genotypes (CC-FADS1 and GG- FADS2) were associated with increased levels of various pro-inflammatory cytokines such as IL-6 as well as decreased levels of those with immunomodulatory activity (IL-RA and CD40L).

Conclusion: rs174547 and rs1535 genetic variants may have a protective effect against omega-6 derived inflammation and an immunomodulatory role on cytokine pathways in obese individuals.

POSTER 100

N-ACYL TAURINES ARE REGULATORS OF PUFA LEVELS AND WHOLE-BODY METABOLISM

Samuel A.J. Trammell, Matthew P. Gillum, TRISHA J. GREVENGOED

Ved Amagerbanen 37B, 2th Copenhagen S, 2300 Denmark

Metabolism of lipids goes far beyond energy storage and membrane synthesis. Many lipid metabolites contribute to regulation of whole body disease and can positively or negatively affect disease states. We study one such class of lipid metabolites, the N-acyl taurines (NATs), which are conjugates of a fatty acid and taurine. These FAAH-hydrolyzed lipids can directly stimulate GLP-1 secretion through receptor activation and indirectly increase this incretin through slowing lipid absorption, leading to improved insulin sensitivity and lower hepatic lipid accumulation. We recently identified the synthase for these lipids, in order to study the necessity of NATs in normal metabolism and disease. We have found that humans synthesize NATs in the liver and in response to dietary supplementation of specific fatty acids. We are also investigating the role of a specific NAT in hepatic inflammation and lipid accumulation and preliminary results are promising for a specific NAT. Understanding regulation and necessity of these lipids will both improve our basic understanding of metabolism and potentially provide novel pathways to target in treatment of metabolic disease.

POSTER 101

EFFECTS OF LIPOPROTEIN APHERESIS AND EVOLOCUMAB TREATMENT ON ESSENTIAL FATTY ACIDS AND LIPID MEDIATORS

CHAOXUAN WANG[1,2], Anne Kaufmann[3], Nadine Rohwer[1,2,4], Nils H Schebb[3] and Karsten H Weylandt[1,2]

1) Medical Department B, Division of Hepatology, Gastroenterology, Oncology, Hematology, Palliative Care, Endocrinology and Diabetes, University Hospital Ruppin-Brandenburg, Brandenburg Medical School, Neuruppin, Germany
2) Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology, Brandenburg Medical School and University of Potsdam, Potsdam, Germany
3) University of Wuppertal, Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, Wuppertal, Germany
4) Department of Molecular Toxicology, German Institute of Human Nutrition, Potsdam, Germany

Cardiovascular disease (CVD) is a disease of the cardiac and circulatory system. Dyslipidemia is a major risk factor for cardiovascular disease. Blood lipids, and in particular low-density lipoprotein (LDL) cholesterol, play an important role in the development of cardiovascular disease. Lipoprotein apheresis (LA) and evolocumab are two highly potent treatments to lower LDL cholesterol, but there is still little known about the effects of these two treatments on fatty acids and lipid mediators.

We analyzed blood samples from 37 patients receiving different treatments. Patients were stratified according to receiving LA treatment (n=19) and evolocumab treatment (n=18). Serum FA analysis was performed by gas chromatography. Lipid mediator analysis was done by liquid-chromatography with tandem mass spectrometry (LC-MS/MS).

We were able to show that evolocumab, as compared to LA treatment, significantly lowered arachidonic acid (AA) levels, but not eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) levels in the plasma. Furthermore, there was a trend towards lower levels of a variety of lipoxygenase- as well as cyclooxygenase- and cytochrome P450 enzyme-derived lipid mediators in evolocumab treated patients, with
EPA-derived (and possibly anti-inflammatory) 15-hydroxyeicosapentaenoic acid (15-HEPE) showing a trend towards an increase due to evolocumab treatment.

This work was supported by a Grant from Amgen.

POSTER 102

HEPATIC STEATOSIS AND FIBROSIS, FADS1 GENOTYPE AND FATTY ACID COMPOSITION

Miriam Rabehl[1,2], CHAOXUAN WANG[1,2], Yanan Xiao[1,2], Can G Leineweber[1,2], Anne Pietzner[1,2], Michael Rothe[3], Adelheid Jung[1] and Karsten H Weylandt[1,2]

1) Medical Department B, Division of Hepatology, Gastroenterology, Oncology, Hematology, Palliative Care, Endocrinology and Diabetes, University Hospital Ruppin-Brandenburg, Brandenburg Medical School, Neuruppin, Germany
2) Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology, Brandenburg Medical School and University of Potsdam, Potsdam, Germany
3) Lipidomix, Berlin, Germany

Previous studies have indicated that activity of fatty acid desaturases (FADS), namely FADS1, is involved in cardiometabolic risk.

While initial studies in patients with coronary artery disease indicated increased risk of atherosclerosis with genotypes leading to higher levels of FADS1 activity, other studies indicate that instead the knock-down of FADS1 expression confers increased cardiometabolic risk. Several human studies support this finding and have reported an association between genotypes with decreased FADS1 activity and obesity, nonalcoholic fatty liver disease (NAFLD) and other metabolic disturbances.
We assessed FADS1 genotype and fatty acid profiles in a cohort of 85 patients with NAFLD. In this cohort we were able to confirm previous observations with significantly different blood fatty acid profiles depending on FADS1 genotype. Patients with lower FADS1 activity as indicated by delta-5-desaturase index and rs174556 genotype had higher levels of fibrosis as measured by noninvasive Fibroscan measurements. Further analyses are now on-going to further characterize lipidomic and clinical characteristics in this cohort.

POSTER 103

SHORT-TERM CALORIC RESTRICTION ALTERS THE INFLAMMATORY MICROENVIRONMENT IN THE LIVER OF AGED MICE

MAREIKE WICHMANN, Patrick SchÄdel, Anna Czapka, Nadja Gebert, Alessandro Ori, Oliver Werz

Department of Pharmaceutical/Medicinal Chemistry Philosophenweg 14, 07743 Jena Germany

Aging is often accompanied by an increased pro-inflammatory status leading to chronic, systemic and sterile inflammation promoting many age-related diseases. This process is termed inflammaging. As caloric restriction (CR) - in contrast to high caloric intake - has been implicated to have positive effects on life span and inflammation, we investigated its impact on inflammatory signaling pathways and whether it can alleviate inflammaging mediated by lipid mediators (LM) and cytokines. However, organ-specific effects of short-term CR on inflammatory processes, in particular in the context of LM signaling, remain elusive. Here, we provide an extensive assessment of the influence of age on the hepatic proteome, cytokine secretome and metabololipidome and to what extent they can be altered in 18-month-old mice by short-term CR (4 weeks). Additionally, we tested if the CR-mediated effects can be maintained after a re-feeding period, by allowing ad libitum food intake for two days after initial CR. Surprisingly, our results show that aging did not lead to a significant increase in levels of pro-inflammatory LMs like prostaglandins or leukotrienes within the liver of aged mice. Further, CR failed to revert the metabolipidome back to levels of young animals. Instead, we found a pronounced increase in monohydroxylated precursors while the majority of both bioactive prostanoids and specialized pro-resolving mediators (SPM) were substantially reduced following CR. Interestingly, re-feeding did not reverse the observed CR effects but rather compromised the metabololipidome even further. Our findings reveal that short-term limitation of food intake is not able to restore the metabololipidome back to juvenile levels within the liver of aged mice.

Together, we shed light on the need of accounting for both, beneficial and detrimental effects of nutritional intervention to aid in therapeutic approaches towards inflammation and disease in the elderly.

POSTER 104

COMBINED SAMPLE PREPARATION AND LC-MS BASED ANALYSIS OF OXYLIPINS AND THEIR PRECURSOR FATTY ACIDS IN BIOLOGICAL SAMPLES

MICHELLE WIEBEL, Elisabeth Koch, Nadja Kampschulte, Nils Helge Schebb

Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Gaussstr. 20, 42119 Wuppertal, Germany

Eicosanoids and other oxylipins are potent lipid mediators which are involved in the regulation of numerous physiological processes and therefore of high interest in biological samples. Modern analytical methods used for the quantification of oxylipins in biological samples are largely based on liquid chromatography-mass spectrometry (LC-MS). However, the precursor polyunsaturated fatty acids (PUFA) are serval orders of magnitude higher concentrated in biological samples compared to the oxylipin levels. Thus, a simultaneous quantification of PUFA is hardly possible using a single method.
Here, we developed a method that allows the parallel quantification of fatty acids and oxylipins from a single sample with a combined sample preparation. Biological samples are prepared by protein precipitation using 2-propanol. An aliquot of the supernatant is diluted in ethanol for the LC MS-based analysis of non-esterified fatty acids and the remaining supernatant is hydrolyzed with potassium hydroxide in methanol/water and neutralized with acetic acid. An aliquot of this solution is directly analyzed by LC-MS for total fatty acid analysis, while oxylipins are analyzed by LC-MS from the remaining solution after solid-phase extraction (Talanta, 2020, 217, 121074).

For quantification of fatty acids, a targeted LC-MS method for 41 fatty acids and 11 isotopically labeled fatty acids was developed (Anal. Bioanal. Chem. 2021, 413, 5439-5451). Chromatographic separation was carried out within a run time of 15 min using a C8 reversed-phase column and water/acetonitrile/methanol/acetic acid (solvent A) and acetonitrile/methanol/acetic acid (solvent B) as eluents. The mass spectrometric detection was performed in pseudo scheduled reaction monitoring mode following negative electrospray ionization.

The developed method can be used for different biological samples such as cells, tissue or plasma and allows a reliable quantification of the (oxidized) fatty acids. A comparison of the determined total fatty acid levels in plasma by means of LC-MS with gas chromatography flame ionization standard methods revealed high accuracy of the developed method (agreement 80-120%). In addition, interday and intraday variances < 15% were observed underlining the good precision of the method.

POSTER 105

EICOSANOID METABOLISM IN THE CONTEXT OF ILC EFFECTOR PHENOTYPE AND LINEAGE

LORENZ WIRTH, Johan Kolmert, Chris Tibbit, Whitney Weigel, Katerina Pardali, Craig Wheelock, Apostolos Bossios, Thomas Hochdörfer, Sven-Erik Dahlén, Jenny Mjösberg

Lidköpingsvägen 19, 12139 Johanneshov, Sweden

Group 2 innate lymphoid cells (ILC2) function is heavily regulated by members of the eicosanoid family of lipid mediators. Depending on the species, eicosanoids exert potent activating- or inhibitory function, as seen for the prostanoids prostaglandin D_2 (PGD_2) and PGE_2, respectively. Moreover, in the context of cytokine stimulation, human ILC2 produce endogenous PGD_2, which they require for their activation. Eicosanoid metabolism and function across human ILC subsets, however, remains poorly understood.

The aim of our study was to delineate this aspect of human ILC biology. Analysis of existing RNA-seq data of ILC subsets revealed several transcripts involved in eicosanoid-synthesis, that were previously not associated with ILC biology. Using mass-spectrometry we confirmed the production of PGD_2 from alarmin-stimulated human tonsil ILC2s. We also detected the production of PGE_2, its downstream metabolite PGB_2, and PGE_1, suggesting an active PGE_2-synthesis. We additionally detected PGE_2- production from human tonsil ILC3, at levels higher than for human ILC2. Exogenous PGE_2 had a stimulatory function on human ILC3, promoting type 3 cytokine production. These data suggest analogous functions of PGD_2 on ILC2 and PGE_2 on ILC3, and a dichotomic effect of PGE_2 on ILC2 and ILC3, raising the possibility for a role of prostanoids in ILC2-ILC3 plasticity.

Understanding the role of endogenous and exogenous prostanoids in ILC regulation has important therapeutic implications for conditions such as allergy and asthma.

POSTER 106

OXYLIPIN CHANGES DURING CHEMOTHERAPY AND CHECKPOINT INHIBITOR THERAPY

JONAS WOERDEMANN[1,2], Chaoxuan Wang[1,2], Anne Pietzner[1,2], Michael Rothe[3], Inci Dogan[3], Nadine Rohwer[1,2,4], Andreas Loew[1] and Karsten H Weylandt[1,2]

1) Medical Department B, Division of Hepatology, Gastroenterology, Oncology, Hematology, Palliative Care, Endocrinology and Diabetes, University Hospital Ruppin-Brandenburg, Brandenburg Medical School, Neuruppin, Germany
2) Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology, Brandenburg Medical School and University of Potsdam, Potsdam, Germany
3) Lipidomix, Berlin, Germany
4) Department of Molecular Toxicology, German Institute of Human Nutrition, Potsdam, Germany

Immune checkpoint (ICI) therapy has an increasingly important role as antitumor therapy for various tumor entities. Lipid mediators have an important role in the modification of immune responses.

The aim of this pilot study was to assess effects of treatment regimens for lung cancer consisting of chemotherapy as well as immune checkpoint inhibitor therapy on blood content of essential fatty acids as well as lipid mediators.

A total of eleven patients receiving chemotherapy plus ICI therapy for lung carcinoma were recruited. Blood samples were taken from each patient, before initiation of the treatment cycle, immediately after administration of compounds, and 24 hours after treatment.

There were no significant changes in fatty acid composition of blood cells before and after treatments. In contrast, we found relevant differences in levels of oxylipins. In particular, several epoxyeicosatrienoic acid (EET) compounds were significantly reduced after treatments.

POSTER 107

DOES HUMANIZATION OF THE REACTION SPECIFICITY OF ALOX15 AND ALOX15B IMPACT ATHEROGENESIS IN APOE-DEFICIENT MICE.

JIAXING YANG, Astrid Borchert and Hartmut Kuhn

Author Address: Department of Biochemistry, Charite - University Medicine Berlin, Chariteplatz 1, 10117 Berlin, Germany

Arachidonic acid lipoxygenase isoforms (ALOX) have been implicated in atherogenesis. Although ALOX15 expression was minimal in advanced human lesions specific ALOX15 products have been detected in human and rabbit plaques. ALOX15 and ALOX15B occur in human lesions implicating these enzymes in human atherogenesis. When the Alox15-/- gene was functionally inactivated in apoE-/- and LDL-R-/- mice the animals were protected from lipid deposition in the arterial wall. These data suggested a pro-atherogenic role of the arachidonic acid 12-lipoxygenating mouse Alox15. In contrast, when human ALOX15 was overexpressed in mice and rabbits an anti-atherogenic effect of the transgene was observed. In fact, transgenic ALOX15 overexpressing animals were protected from lipid deposition in the arterial wall and this effect was related to an increased formation of anti-inflammatory and/or pro-resolving mediators. The molecular basis for the controversial effects observed with the Al ox15-/- and the transgenic ALOX15 overexpressing animals remain unclear but the different reaction specificities of mouse (arachidonic acid 12-lipoxygenating) and human (arachidonic acid 15-lipoxygenating) ALOX15 may contribute.

To explore the hypothesis that the reaction specificity of Alox15, Alox15b and Alox5 is important for the potential role of these enzymes in mouse atherosclerosis models, we created three different genetically modified mouse strains expressing Alox-isoforms with altered reaction specificity: i) Alox15 knock-in mice (Alox15-KI) expressing an Alox15 mutant (L353F) with humanized reaction specificity (arachidonic acid 15-lipoxygenating) instead of the arachidonic acid 12-lipoxygenating wildtype enzyme. ii) Alox15b knock-in mice (Alox15b-KI) expressing an Alox15b mutant (Y603D+H604V) with humanized reaction specificity (arachidonic acid 15-lipoxygenating) instead of the arachidonic acid 8-lipoxygenating wildtype enzyme. iii) Alox5 knock-in mice (Alox5-KI) expressing an Alox5 mutant (F359W+A424I+N425M) exhibiting a dominant arachidonic acid 15-lipoxygenating activity. Homozygous allele carriers are currently cross with apoE-/- mice and individuals carrying the knock-out/knock-in alleles at both gene loci will be selected. As control groups individuals carrying the knock-out allele at the apoE locus but the wildtype allele at the Alox15, Alox15b or Alox5 loci will be bred out. The aortic lipid deposition will be quantified by conventional morphometric methods and by HPLC analyses of the tissue concentration of free cholesterol and different cholesterol esters.

POSTER 108

INVESTIGATING A MODULATION OF THE ARACHIDONIC ACID CASCADE IN HUMAN MACROPHAGES BY DRUGS AND PHYTOCHEMICALS

LILLI ZINNERT, Rebecca Pfaff, Nicole M. Hartung and Nils Helge Schebb

Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Gaussstrasse 20, 42119 Wuppertal, Germany

The arachidonic acid (ARA) cascade and its lipid mediators play a key role in inflammatory processes. Especially the products of cyclooxygenases (COX) and lipoxygenases (LOX) generate well described highly potent oxylipins. Established pharmaceuticals such as nonsteroidal anti-inflammatory drugs (NSAIDs) relieve pain, fever and inflammation intervening in these enzymatic pathways. Moreover, several secondary plant metabolites are described to have anti-inflammatory effects, which might be caused by a modulation of the ARA cascade.

Here, we investigate the modulation of the ARA cascade in differentiated (Vit-D3, TGF-beta1) THP-1 cells and human blood derived monocytes differentiated into M1-like macrophages using CSF-2 + IFNgamma and M2-like macrophages using CSF-1 and IL-4 with and without stimulation with bacterial lipopolysaccharide (LPS). The effects of specific and non-specific pharmaceuticals on oxylipin pattern and enzyme abundance were quantified by LC-MS based targeted metabolomics and targeted proteomics analysis.

In order to comprehensively characterize modulatory effects of ARA cascade, we intensely tested specific COX and LOX inhibitors in differentiated THP-1 cells and primary macrophages.

Nearly complete blockage of COX-derived oxylipin formation was achieved using indomethacin which inhibits both COX-1 and COX-2 activity. Partial inhibition of COX-derived oxylipins such as PGE2 was achieved using celecoxib, a selective COX-2 inhibitor. Thus, selectivity of the used COX-inhibitors is strongly related to the resulting oxylipin patterns. Moreover, COX-2 activity and abundance was decreased in a dose-dependent manner using dexamethasone.

Investigating the LOX pathways, 5-HETE formation was blocked dose-dependently without affecting 5-LOX enzyme abundance using PF4191834 in THP-1 cells. Similarly, 15-LOX activity was inhibited by ML351 reflected in reduced concentrations of 15-LOX oxylipin products in primary M2-like macrophages.

Targeted metabolomics and proteomics analysis of human macrophages allows the detailed investigation of the effect of a pharmaceutical modulation of the ARA cascade: The selectivity, site effects on other enzymes as well as a shunt towards other pathways of the ARA cascade can be depicted. This provides the possibility to monitor effects of new drugs and phytochemicals on macrophages and the ARA cascade in detail. Based on this opportunity new substances will be characterized for modulation of ARA cascade enzymes and further anti-inflammatory potential.

POSTER 109

QUANTITATIVE DETERMINATION OF EICOSANOIDS AND STEROID METABOLITES IN URINE FOR CLINICAL PHENOTYPING OF ASTHMA

JAVIER ZURITA PEREZ*, Johan Kolmert*, Julia Sieminska**, Craig E. Wheelock*

* Unit of Integrative Metabolomics, Institute of Environmental Medicine; Karolinska
** Metabolomics Laboratory, Clinical Research Centre, Medical University of Bialystok, Poland

Eicosanoids are signaling lipids involved in many respiratory and circulatory diseases such as asthma and atherosclerosis as well as neurodegenerative diseases including Alzheimer (Dennis and Norris, 2015; Herbst-Robinson et al., 2015). These lipid mediators are rapidly metabolized and excreted through urine, thus making this biofluid a useful analytical target to monitor their systemic release and turnover rate. Of significant interest, eicosanoids are 20 carbon-length lipids arising from the metabolism of arachidonic acid and have been extensively studied as targets for treatment of inflammatory diseases. Particularly, LTE4 and 2,3-dinor-11-beta-PGF2a, metabolites of the cysteinyl leukotriene and PGD2 pathway respectively, have been shown to correlate well with asthma severity characterized by type 2 inflammation (Kolmert et al., 2021). In addition, linoleic acid-derived lipids, such as 9,10-diHOME and 12,13-diHOME, have recently gained interest due to their elevation in feces of neonates at high risk of developing asthma (Levan et al., 2019). Common treatments for asthma management include inhaled and/or oral corticosteroids. Herein, we present an integrated urinary lipid and drug metabolite platform that is able to determine the concentration of 22 oxylipins from arachidonic acid, linoleic acid and DHG-Linolenic acid, and 15 parent drug/or drug metabolites including COX-inhibitors and synthetic as well as endogenous steroids (e.g., prednisolone and cortisol). The analytes are extracted from urine using an automated 96-well format, making the method suitable for analysis of large clinical cohorts. Limit of quantification ranges from 0.03-0.3 ng/mL for the oxylipin metabolites with a linear range of 0.11-176 ng/mL on average. For the drug panel, the limit of quantification ranges from 0.06-120 ng/mL with a linear range of 0.011-0.90 ug/mL on average. Furthermore, the analysis of more than 2000 urine samples over eight weeks evidenced a precision of 3-17 % coefficient of variation (CV) during the whole data acquisition period (drug panel 9-29 %CV). Overall, 17 out of 20 urinary eicosanoids were reported in more than 90% of the samples analyzed, and prednisolone was detected in 82%. This method will be useful for large-scale investigation of the role of eicosanoid metabolism in health and disease.

- Dennis, E.A. and Norris, P.C. (2015), Eicosanoid storm in infection and inflammation, Nature Reviews Immunology, 15(8), pp. 511 -523. doi:10.1038/nri3859.
- Herbst-Robinson, K.J. et al. (2015), Inflammatory Eicosanoids Increase Amyloid Precursor Protein Expression via Activation of Multiple Neuronal Receptors, Scientific Reports, 5(December), pp. 1-16. doi:10.1038/srep18286.
- Kolmert, J. et al. (2021), Urinary Leukotriene E4 and Prostaglandin D2 Metabolites Increase in Adult and Childhood Severe Asthma Characterized by Type 2 Inflammation, American Journal of Respiratory and Critical Care Medicine, 203(1), pp. 37-53. doi:10.1164/rccm.201909-1869OC.
- Levan, S.R. et al. (2019), Elevated faecal 12,13-diHOME concentration in neonates at high risk for asthma is produced by gut bacteria and impedes immune tolerance, Nature Microbiology, 4(11), pp. 1851-1861. doi:10.1038/s41