LIPID MAPS MEETING POSTER SESSIONS

The LIPID MAPS meeting features two poster sessions with 55 posters. Of these posters, four were selected for short talks this year (see program). We received so many high quality, relevant abstracts that making these selections was very hard. We will be sure to include additional short talk slots in the program for our 2011 meeting. Please use the list of posters, the map that follows, and the abstracts to navigate the posters sessions.

1. **Deletion of host acyl-coA binding protein alters membrane curvature and inhibits genomic replication of a positive-strand RNA virus**
   Xiaofeng Wang, Arturo Diaz, Lan Mao, Paul Ahlquist (Texas AgriLife Research and Texas A&M University).
2. **N-modification of phosphatidylethanolamine by γ-ketoaldehydes induces HUVEC activation**
   Sean S. Davies, Lilu Guo, Zhongyi Chen, Blake Sullivan, Venkataraman Amarnath (Vanderbilt University).
3. **Lipophilic regulators of Hedgehog signaling**
   Navdar Sever, Randall K. Mann, Helena Khaliullina, Suzanne Eaton, Philip A. Beachy (Stanford University).
4. **Restriction of inflammatory response by a novel series of electrophilic oxidation products of phospholipids: implication in chronic granulomatous disease (CGD)**
   Huiyong Yin, Wei Han, Konjeti R. Sekhar, Michael L. Freeman, Timothy S. Blackwell, (Vanderbilt University School of Medicine).
5. **Quantitation of membrane-bound protein-ligand interactions using backscattering interferometry**
   Michael M. Baksh, Amanda Kussrow, Mauro Mileni, M.G. Finn, Darryl Bornhop (Vanderbilt University).
6. **Eicosanoid profiles in the K/BxN serum transfer model**
   Anna M. Ritzman, Darren S. Dumlao, Edward A. Dennis, Charles R. Brown (University of Missouri-Columbia).
7. **Diacylglycerol kinase epsilon specificity for phosphatidic acid or diacylglycerol is dependent on both their sn-1 and sn-2 acyl chains**
   Michael Lung, Yulia Shulga, Pavlina Ivanova, David Myers, Stephen Milne, H. Alex Brown, Matthew K. Topham, Richard M. Epand (Vanderbilt University School of Medicine).
8. **Atypical lipid species in macrophages**
   Pavlina T. Ivanova, Stephen B. Milne, H. Alex Brown (Vanderbilt University School of Medicine).
9. **Capture and release of alkyne-derivatized glycerophospholipids using cobalt chemistry**
   Stephen B. Milne, Keri A. Tallman, Remigiusz Serwa, Carol A. Rouzer, Michelle D. Armstrong, Lawrence J. Marnett, Charles M. Lukehart, Ned A. Porter, H. Alex Brown (Vanderbilt University School of Medicine).
10. **Development of high-throughput platforms for the study of drug-membrane interactions**
    Claire Stanley, Duncan Casey, Antony Gee, Andrew deMello, Oscar Ces (Imperial College, UK).
11. **Subcellular membrane lipidomics in TLR 4-challenged macrophages**
    Alexander Y. Andreyev, Eoin Fahy, Ziqiang Guan, Samuel Kelly, Xiang Li, Jeff G. McDonald, Stephen Milne, David Myers, Andrea Ryan, Bonne M. Thompson, Elaine Wang, Yihua Zhao, H. Alex Brown, Alfred H. Merrill, Jr., Christian R.H. Raetz, David W. Russell, Shankar Subramaniam, Edward A. Dennis (University of California, San Diego).
12. **Effects of omega-3 fatty acids on lipid metabolism and signaling using lipidomic analyses**
    Joshua D. Brooks, Paul C. Norris, Edward A. Dennis (University of California, San Diego).
13. **Lipidomic analysis of carrageenan-induced hyperalgesia reveals essential eicosanoid production in the spinal cord**
    Darren S. Dumlao, Matthew W. Buczynski, Camilla I. Svensson, Bethany Fitzsimmons, Jennifer Wirkus, Faith E. Jacobsen, Xiao-Ying Hua, Tony L. Yaksh, Edward A. Dennis (University of California, San Diego).

Presenting author is underlined.

★ *Selected for short talk*

14. **Mitochondrial phosphatase PTPMT1 dephosphorylates phosphatidylglycerol phosphate and is essential for cardiolipin biosynthesis**
Ji Zhang, Ziqiang Guan, Anne N. Murphy, Sandra E. Wiley, Carolyn Worby, James L. Engel, Philip Heacock, Oanh Kim Nguyen, Jonathan Wang, Christian R.H. Raetz, William Dowhan, Jack E. Dixon (University of California, San Diego).

15. **Prostaglandin D2 enhances interleukin-1beta-induced cyclooxygenase-2 expression in osteoarthritic cartilage POSTER NOT PRESENTED**
Nadia Zayed, Nadir Chabane, Fatima Ezzahra El Mansouri, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Hassan Fahmi (Notre-Dame Hospital and University of Montreal, Canada).

16. **Valproic acid suppresses interleukin-1β-induced microsomal prostaglandin E2 Synthase-1 expression in chondrocytes POSTER NOT PRESENTED**
Nadia Zayed, Nadir Chabane, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Hassan Fahmi (Notre-Dame Hospital and University of Montreal, Canada).

17. **Interleukin-1-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in human OA chondrocytes is associated with histone H3K4 methylation POSTER NOT PRESENTED**
Fatima Ezzahra El Mansouri, Nadia Zayed, Nadir Chabane, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Hassan Fahmi (Notre-Dame Hospital and University of Montreal, Canada).

18. **Myeloid differentiation primary response protein 88 couples reverse cholesterol transport to inflammation**★
Kathleen A. Smoak, Jim J. Aloor, Jennifer Madenspacher, B. Alex Merrick, Jennifer B. Collins, Xuewei Zhu, Giorgio Cavigiolio, Michael N. Oda, John S. Parks, Michael B. Fessler (National Institute for Environmental Health Sciences).

19. **The different gene profiles by oxysterols in macrophage**
Norihito Shibata, Bonne M. Thompson, Ruth Yu, Ronald M. Evans, David W. Russell, Christopher K. Glass (University of California, San Diego).

20. **A novel role of phospholipids in brain prostanoid level regulation**
Colin K. Combs, Eric J. Murphy, Mikhail Y. Golovko (University of North Dakota).

21. **Lipid profiling of tumor lipid droplets by UPLC/TOF MS**
Hayley Crowe, John P. Shockcor, Ladan Mirbahai, Martin Wilson, Christopher S. Shaw, Carmel McConville, Roger D.G. Malcomson, Risto A. Kauppinen, Andrew C. Peet, Julian L. Griffin (University of Cambridge, UK).

22. **Heat Shock Protein 70B' (HSP70B') expression and release in response to human oxidized LDL immune complex in macrophages**
Kent J. Smith, Waleed O. Twal, Farzan Soodavar, Gabriel Virella, Maria F. Lopes-Virella, Samar M. Hammad (Medical University of South Carolina).

23. **Embryonic expression of leukotriene biosynthetic proteins**
Tobias Strid, Hong Qian, Cecilia Karlsson, Mats Söderström, Mikael Sigvardsson, Sven Hammarström (Linköping University, Sweden).

24. **Sterol modified lipids-non-exchangeable lipids for stable lipid vesicle formation**
Francis C. Szoka, Zhaohua Huang (University of California San Francisco).

25. **Saturated fatty acid lauric acid activates Toll-like receptor 4 by inducing dimerization and recruitment of the receptor into lipid rafts in a ROS dependent manner**
Scott W. Wong, Myung-Ja Kwon, Ryan Snodglass, Augustine M.K. Choi, Hong-Pyo Kim, Kiichi Nakahira, Daniel Hwang (Western Human Nutrition Research Center/ARS/USDA and University of California Davis).

26. **Functional analysis of lysoPI acyltransferase 1 (LPIAT1)**
Hiroyuki Arai, Takao Inoue (University of Tokyo and CREST JST, Japan).

27. **Identification of key residues determining species differences in inhibitor binding of Microsomal Prostaglandin E Synthase 1**
Sven-Christian Pawelzik, Narasimha Rao Uda, Linda Spahiu, Caroline Jegerschöld, Patric Stenberg, Hans Hebert, Ralf Morgenstern, Per-Johan Jakobsson (Karolinska Institutet, Sweden).

28. **Investigating neurodegenerative disorder systems using USPIO-nanoparticles with (SECARS) and stimulated raman scattering microscopy** **POSTER NOT PRESENTED**
Lina H. Machtoub (Innsbruck Medical University, Austria).

29. **Stability and analysis of eicosanoids and docosanoids in culture media**
Krishna Rao Maddipati (Wayne State University).

30. **Metabolomic and genomic analysis of cafeteria-diet induced macrophage infiltration of adipose tissue reveals a robust model of obesity, inflammation and glucose intolerance**
Brante P. Sampey, Matt Wilkerson, Amanda M. Vanhoose, Helena Winfield, Olga Ilkayeva, Michael J. Muehlbauer, Deborah M. Muoio, Chris B. Newgard, David Neil Hayes, Liza Makowski (University of North Carolina at Chapel Hill).

31. **Computational analysis of data from LC ESI-MS/MS of sphingolipids using a peak detection algorithm that eliminates manual peak detection and integration**
David Goldberg, Samuel Kelly, Elaine Wang, M. Cameron Sullards, Alfred H. Merrill, Jr. (Georgia Institute of Technology).

32. **Quantitative analysis of traditional ceramides (N-acyl-sphingoid bases) and 1-deoxy-ceramides (N-acyl-1-deoxy- and -1-desoxymethyl-sphingoid bases) by reverse phase liquid chromatography electrospray ionization tandem mass spectrometry**
Sibali Bandyopadhyay, Hyejung Park, M. Cameron Sullards, Alfred H. Merrill, Jr. (Georgia Institute of Technology).

33. **Sphingolipidomic analysis of NIST human plasma**
Kristin N. Jones, Rebecca L. Shaner, Elaine Wang, Samuel L. Kelly, Sibali Bandyopadhyay, M. Cameron Sullards, Alfred H. Merrill, Jr. (Georgia Institute of Technology).

34. **Age-dependent changes in the sphingolipid composition of CD4+ T cell membranes and immune synapses**
Zhaofeng Huang, Melissa G. Marko, Alberto Molano, Stephen C. Bunnell, Dayong Wu, Alfred H. Merrill, Jr., Simin Nikbin Meydani (Tufts University).

35. **Oxidized cholesteryl esters activate macrophage via toll-like receptor-4**
Soo-Ho Choi, Richard Harkewicz, Felicidad Almazan, Yury I. Miller (University of California, San Diego).

36. **Oxidized cholesteryl esters and phospholipids in zebrafish fed a high-cholesterol diet: Macrophage binding and activation**
Longhou Fang, Richard Harkewicz, Karsten Hartvigsen, Tiffany Sayaphupha, Edward A. Dennis, Joseph L. Witztum, Sotirios Tsimikas, Yury I. Miller (University of California, San Diego).

37. **Mapping the *M. tuberculosis* lipidome reveals previously unknown deoxymycobactins***
Sunhee Hong, Cressida A. Megan, Tan-Yun Cheng, David Young, Emilie Layre, Lindsay Sweet, John Annand, Rahul S. Talekar, Matthew McConnell, Anthony C. Debono, Isamu Matsunaga, D. Branch Moody (Brigham and Women's Hospital and Harvard Medical School).

38. **Determination of oxidized neutral lipids in human atherosclerosis**
Patrick M. Hutchins, Robert M. Barkley, Robert C. Murphy (University of Colorado Denver).

39. **PAR signaling in platelets activates cPLA$_2$-α differently for COX-1 and 12-LOX catalysis**
Olivier Boutaud, Michael Holinstat, Heidi E. Hamm, John A. Oates (Vanderbilt University School of Medicine).

40. **Discovery of novel non-cytidine liponucleotides in *S. cerevisiae* and *E. coli***
Gregory M. Laird, Ziqiang Guan, Reza Kordestani, Christian R.H. Raetz (Duke University Medical Center).

41. **Exploring lipid-protein interactions: identification of endogenous ligands of CD1**
Ziqiang Guan, Neil Haig, Xiao-ning Xu, Andrew J. McMichael, Christian R. H. Raetz (Duke University Medical Center).

42. **Measurement of bioactive lipids using immunoassays and receptor interaction methodologies**
Kelli Moreno, Jon Wojciak, Rosalia Matteo, Mary-Ann Campbell, Roger Sabbadini (Lpath Inc.).

43. **Characterization of an ASAH1-knockdown H295R stable cell line: A role for nuclear ASAH1 in steroidogenic gene transcription**

Natasha C. Lucki, Marion B. Sewer (Georgia Institute of Technology).

44. Searching the LIPID MAPS databases
Dawn Cotter, Eoin Fahy, Manish Sud, Shankar Subramaniam (University of California, San Diego).

45. Tools for lipidomics: mass spectrometry prediction and structure drawing methods
Dawn Cotter, Eoin Fahy, Manish Sud, Shankar Subramaniam (University of California, San Diego).

46. LIPID MAPS methodologies for pathway construction and analysis
Robert Byrnes, Dawn Cotter, Eoin Fahy, Shakti Gupta, Sean Li, Mano Maurya, Manish Sud, Shankar Subramaniam (University of California, San Diego).

47. A strategy for the quantitative analysis of all lipids with a single acquisition method
Sahana Mollah, Brigitte Simons, Stephen Tate (AB SCIEX, Canada).

48. Lipidomic analysis of bioactive lipids in Chronic Lymphocytic Leukaemia
Mojgan Masoodi, David Spaner, Albert Koulman, Michael Eiden, Dietrich A. Volmer (Medical Research Council, UK).

49. Profiling and imaging of phospholipids in rat brain sections after ischemic stroke*
Hay-Yan J. Wang, Cheng Bin Liu, Hsuan-Wen Wu (National Sun Yat-Sen University, Taiwan).

50. In situ desalting that assists the direct MALDI-MS profiling of tissue phospholipids
Hay-Yan J. Wang, Hsuan-Wen Wu, Cheng Bin Liu, Hsuan-Po Liu (National Sun Yat-Sen University, Taiwan).

51. Quantitative proteomic analysis of the effect of 24(S),25-epoxycholesterol on neuronal cells
Ian R. Gilmore, Jenny Ho, Martin Hornshaw, William J. Griffiths, Yuqin Wang (Swansea University, UK).

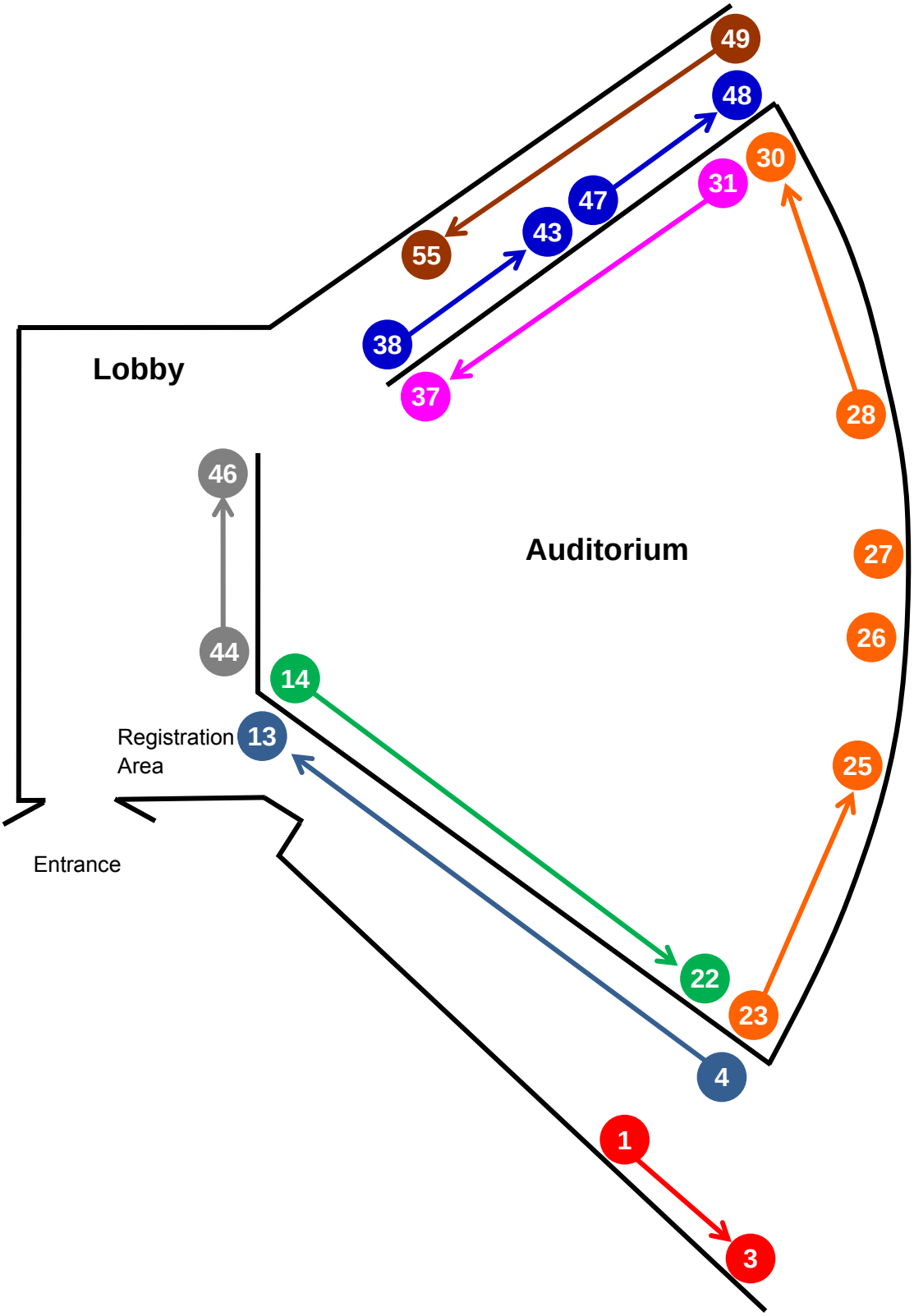
52. Analysis of oxysterols and cholestenoic acids in plasma and CSF by charge-tagging and LC-MS^{n*}
William J. Griffiths, Michael Ogundare, Andrew Lockhart, Jan Sjövall, Yuqin Wang (Swansea University, UK).

53. SIMS and MALDI imaging of glycerophospholipid
Melissa K. Passarelli, Nicholas Winograd (The Pennsylvania State University).

54. Effects of inflammatory cytokines on sulfoglucuronosyl paragloboside expression in human cerebromicrovascular endothelial cells
Robert K.Yu, Somsankar Dasgupta, Guanghu Wang (Medical College of Georgia).

55. Inhibition of sodium ion channel activity by anti-lipid A antibody of chicken with campylobacteriosis
Robert K. Yu, Seigo Usuki, Yoshihiko Nakatani, Kyoji Taguchi, Toshio Ariga (Medical College of Georgia).

POSTER SESSION MAP



1. Deletion of host acyl-coA binding protein alters membrane curvature and inhibits genomic replication of a positive-strand RNA virus

Xiaofeng Wang[1], Arturo Diaz[2], Lan Mao[1], Paul Ahlquist[2]

[1]Texas AgriLife Research and Department of Plant Pathology & Microbiology, Texas A&M University, Weslaco, TX 78596, [2]HHMI & Institute for Molecular Virology, University of Wisconsin, Madison, WI 53706.

Positive-strand RNA [(+)RNA] viruses are the largest viral class with many important human and animal viruses as well as the great majority of plant viruses. As with all (+)RNA viruses, genomic replication by *Brome mosaic virus* (BMV) occurs on virus-remodeled intracellular membranes. Expression of BMV RNA replication protein 1a markedly increases total fatty acid accumulation and induces invaginations of outer perinuclear ER membranes into the ER lumen to form viral replication compartments, termed as viral spherules. When BMV polymerase $2a^{pol}$ is expressed at a higher level, 1a induces formation of karmellae-like stacks of appressed double-membranes layering around nuclear membranes. Although morphologically distinct, layers and spherules are otherwise functionally equivalent. Acyl-coA binding protein (ACBP) is a highly conserved protein involved in modulating fatty acid synthesis and vesicular trafficking, among other roles. Deletion of ACBP in yeast inhibits synthesis of very-long-chain fatty acids and sphingolipids but increases the synthesis of unsaturated fatty acids. BMV replication is inhibited up to 30-fold upon deletion of ACBP and can be complemented by providing wild type ACBP but not an ACBP mutant that lacks acyl-coA binding activity. Moreover, raft association of BMV 1a and $2a^{pol}$ is not affected despite the decreased sphingolipid synthesis. Conversely, deletion of ACBP resulted in aberrant viral spherules that are smaller but much more abundant than in the wild type strain. Furthermore, smaller and more frequent spherules are formed under conditions that preferentially induce layer formation, suggesting that sharper curvature is favored in this mutant. The inhibition of viral spherule and layer formation is rather specific since karmellae formation is not affected in the mutant strain, suggesting that BMV 1a interacts with specific lipid species or proteins. Further experiments are underway to understand how altered lipid composition affects the formation of the membrane-bound viral RNA replication compartments.

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2. *N*-modification of phosphatidylethanolamine by γ-ketoaldehydes induces HUVEC activation

Sean S. Davies, Lilu Guo, Zhongyi Chen, Blake Sullivan, Venkataraman Amarnath

Division of Clinical Pharmacology and Department of Pharmacology, Vanderbilt University, Nashville, TN.

Levuglandins and isoketals are γ-ketoaldehyde isomers (γKAs) formed by non-enzymatic rearrangement of prostaglandin H_2 and H_2-isoprostanes, respectively. γKAs rapidly modify cellular amines including lysine and phosphatidylethanolamine (PE) to form stable adducts. Increases in γKA protein adduct levels occur in disease conditions associated with inflammation and oxidative stress including atherosclerosis and Alzheimer's Disease. When added to cultured cells, γKAs exert a number of effects that may be relevant to human disease including alterations in ion channel function in myocytes, activation of human umbilical vein endothelial cells (HUVEC) to promote monocyte binding, and induction of cell death in a variety of cell types. While the biological effects of γKAs have been presumed to arise from its adduction to cellular proteins, we recently found that γKAs adduct to PE more rapidly than lysine. Additionally, using isotope dilution LC/MS/MS assays to measure both protein and PE adducts in HUVEC treated with γKA, we found that PE adducts (γKA-PE) were formed in greater abundance than protein adducts. Plasma levels of γKA-PE have also been reported to increase during oxidative stress *in vivo*. To determine if formation of γKA-PE was biologically significant, we treated HUVEC with γKA-PE. γKA-PE induced phosphorylation of p38MAPK, activated HUVEC to bind monocytes, and induced HUVEC death. Thus, γKA-PE is biologically active and mediates at least some of the effects of γKA added to cultured cells. To test whether *N*-modification of PE by any acyl moiety conferred the ability to activate HUVEC, we tested several species of *N*-acyl PE (NAPE). In contrast to γKA-PE, NAPE did not activate HUVEC, and instead inhibited HUVEC activation by LPS or γKA-PE. Thus, γKA-PE may be an important mediator of endothelial cell dysfunction during inflammation and oxidative stress.

3. Lipophilic regulators of Hedgehog signaling

Navdar Sever[1], Randall K. Mann[1], Helena Khaliullina[2], Suzanne Eaton[2], Philip A. Beachy[1]

[1]Department of Developmental Biology, Institute for Stem Cell Biology and Regenerative Medicine, Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California 94305, USA. [2]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany.

The Hedgehog (Hh) signaling pathway plays essential roles in embryonic patterning and adult tissue homeostasis. Stoichiometric binding of the Hh ligand to Patched (Ptc) releases the inhibitory effect of Ptc, leading to activation of Smoothened (Smo). The similarity of Ptc to resistance, nodulation, division (RND) proteins, which export substrates across the bacterial membrane by a proton antiport mechanism, and Smo regulation by small molecule-induced conformational change have led to a model of Ptc function as a transporter in regulating Smo. We found potent sources of lipids that compete with a fluorescent derivative of cyclopamine for binding to Smo and that inhibit Hh signaling in responsive cells. We have purified the activity from Drosophila lipophorin particles and Chlamydomonas flagella using solid phase extraction and reverse phase-HPLC, and are currently analyzing the active species by various mass spectrometric techniques. Unequivocal identification of a physiologic Smo ligand will help the development of novel mechanism-based therapeutics not only for human cancers that arise from mutations in Hh pathway but also for those that have been shown to depend on continuous stimulation with Hh ligand.

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4. Restriction of inflammatory response by a novel series of electrophilic oxidation products of phospholipids: implication in chronic granulomatous disease (CGD)

Huiyong Yin[1-3], Wei Han[1], Konjeti R. Sekhar[4], Michael L. Freeman[4], Timothy S. Blackwell[1,5,6,7]

[1]Departments of Medicine, [2]Pharmacology, [3]Chemistry, [4]Radiation Oncology, [5]Cancer Biology, [6]Cell and Developmental Biology, and [7]Veterans Affairs, Vanderbilt University School of Medicine, Nashville, TN.

Chronic granulomatous disease (CGD) is an inherited disorder of deficiency of NADPH oxidase and characterized by recurrent bacterial and fungal infections and by excessive inflammation. Previous evidence indicates that NADPH oxidase and its downstream reactive oxygen species (ROS) play a critical role in restraining inflammation in animal model of CGD. When challenged with either intratracheal zymosan or LPS, NADPH oxidase-deficient p47*phox-/-* and gp91*phox-/-* mice developed exaggerated and progressive lung inflammation, augmented NF-κB activation, and elevated downstream pro-inflammatory cytokines compared to wild type mice. Replacement of functional NADPH oxidase in bone marrow-derived cells restored the normal lung inflammatory response. Studies *in vivo* and in isolated macrophages demonstrated that in the absence of functional NADPH oxidase, zymosan failed to activate NF-E2-related factor-2 (Nrf)2, a key redox-sensitive anti-inflammatory regulator. We hypothesize that ROS generated from NADPH oxidase cause membrane phospholipid oxidation and generate anti-inflammatory oxidation products. We identified a novel series of potent anti-inflammatory oxidation products derived from free radical-induced oxidation of arachidonate-containing phosphatidylcholine. These compounds contain an electrophilic cyclopentenone moiety which may covalently modify critical cysteine residues in proteins in particular Keap1, the cytosolic sequester of Nrf2. We independently synthesized a representative compound of this class of oxidation product, a 15-deoxy-PGJ_2-containing phospholipid. This compound dose-dependently inhibits NFκB activation in bone marrow-derived macrophage activated by LPS, zymosan, or TNFα. The downstream products of NFκB, such as prostaglandins, are inhibited. Furthermore, this compound also activates Nrf2 pathway. The levels of these compounds are significantly elevated in lung tissues of wild type mice compared to NADPH oxidase-deficient p47*phox-/-* and gp91*phox-/-* mice after intratracheal challenge with LPS or zymosan. These studies support a model in which NADPH oxidase-dependent, redox-mediated signaling may be critical for termination of inflammation by electrophilic oxidation products of membrane phospholipids.

5. Quantitation of membrane-bound protein-ligand interactions using backscattering interferometry

Michael M. Baksh[1], Amanda Kussrow[3], Mauro Mileni[2], M.G. Finn[1], Darryl Bornhop[3]

[1]Department of Chemistry, [2]Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037. [3]Department of Chemistry and Vanderbilt Institute for Chemical Biology, Vanderbilt University, Nashville, TN 37235.

Though membrane-associated proteins are ubiquitous within all living organisms and represent the majority of drug targets, a general method for direct, label-free measurement of ligand binding to native membranes has not been reported. Here we show backscattering interferometry (BSI) to be a viable technique for quantifying ligand-receptor binding affinities in a variety of membrane environments. By detecting minute changes in the refractive index of a solution, BSI allows binding interactions of proteins with their ligands to be measured at picomolar concentrations. It will be shown that equilibrium binding constants in the micromolar to picomolar range can be obtained for small- and large-molecule interactions in both synthetic- and cell-derived membranes without the use of labels or supporting substrates and in free-solution. Three systems will be discussed, a) interactions between Cholera Toxin B (CTB) – Ganglioside GM1; b) three small molecule non-covalent inhibitors with the full-length Fatty Acid Amide Hydrolase membrane associated protein; and c) two agonists, an antagonist and the endogenous ligand with native cell membrane preps of γ-Aminobutyric Acid Receptor B. The simple and low-cost hardware, high sensitivity, and label-free nature of BSI should make it readily applicable to the study of many membrane-associated proteins of biochemical and pharmacological interest.

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6. Eicosanoid profiles in the K/BxN serum transfer model

Anna M. Ritzman[1], Darren S. Dumlao[2], Edward A. Dennis[2], Charles R. Brown[1]

[1]University of Missouri-Columbia, Columbia, MO 65211. [2]University of California, San Diego, La Jolla, CA 92093.

The K/BxN serum transfer model of arthritis is a useful way to study effector-phase mechanisms of arthritis development. Recently, potent pro- and anti-inflammatory lipid mediators called eicosanoids, produced via the metabolism of arachidonic acid by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, have been implicated in playing a large role in arthritic disease. It has been reported that the 5-LOX product, LTB_4, as well as the COX-1 product, PGI_2, are important for K/BxN inflammation. We used a recently developed lipidomic approach to detect the presence and expression profiles of 104 individual eicosanoid species during the development and resolution of K/BxN serum-induced arthritis. C3H/HeJ mice were i.p. inoculated with 150μL of pooled K/BxN serum on day 0 and boosted on day 2 post-inoculation. Arthritis was monitored daily by clinical scoring of the hind and front paws and measuring of tibiotarsal joint swelling. Mice were sacrificed at various time points post-inoculation and ankle joints were taken for analysis. One ankle was used for histological examination and the other was used for lipid extraction. Eicosanoid levels were quantified using liquid chromatography-tandem mass spectrometry. Better understanding the expression of eicosanoids during the progression of arthritic disease may aid in the identification of novel therapeutic targets for this and other inflammatory diseases.

7. Diacylglycerol kinase epsilon specificity for phosphatidic acid or diacylglycerol is dependent on both their *sn*-1 and *sn*-2 acyl chains

Michael Lung[1], Yulia Shulga[1], Pavlina Ivanova[2], David Myers[2], Stephen Milne[2], H. Alex Brown[2], Matthew K. Topham[3], Richard M. Epand[1]

[1]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada. [2]Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. [3]Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah 84112, USA.

The phosphatidylinositol (PtdIns) cycle is a major intracellular signaling pathway mediating a variety of cellular processes. A major player in this pathway is diacylglycerol kinase epsilon (DGKε). DGKε possesses the simplest primary structure of all mammalian DGK isoforms, yet is the only isoform that is inhibited by its product phosphatidic acid (PA) and that shows substrate specificity for *sn*-2 arachidonate-diacylglycerol (DAG). Here we show that human DGKε is selective for both the *sn*-1 and *sn*-2 acyl chains of PA or DAG, preferring the most prevalent acyl chain composition of PtdIns cycle lipids. The PA acyl chain specific inhibition of DGKε is competitive and is not affected by the deletion of DGKε's hydrophobic segment and cationic cluster. Thus, not only is the lipid headgroup being recognized by DGKε's active site, but also a combination of the two acyl chains in PA or DAG. We propose a mechanism of DGKε regulation where its dual acyl chain specificity is used to negatively regulate its enzymatic activity in a PtdIns lipid signaling cycle dependent manner.

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8. Atypical lipid species in macrophages

Pavlina T. Ivanova, Stephen B. Milne, H. Alex Brown

Departments of Pharmacology, Biochemistry, Chemistry, & Vanderbilt Institute for Chemical Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

A large scale profiling and analysis of glycerophospholipid species in macrophages has facilitated the identification of several rare and atypical glycerophospholipid species. By using LC/MS/MS and comparison of the elution and fragmentation properties of these lipids to synthetic standards we were able to identify an array of ether-linked phosphatidylinositols (PI), phosphatidic acids (PA), phosphatidylserines (PS), very long chain phosphatidyl-ethanolamines (PE) and phosphatidylcholines (PC) as well as phosphatidylthreonines (PT) and a wide collection of odd carbon fatty acid-containing phospholipids in macrophages. A comprehensive qualitative analysis of glycerophospholipids from different macrophage cells was conducted. During the phospholipid profiling of the macrophage-like RAW 264.7 cells, we identified dozens of rare or previously uncharacterized phospholipids, including ether-linked PI, PS and PA; the phospholipid PT; and very long polyunsaturated forms of PC and PE. Additionally, large numbers of phospholipids containing at least one odd carbon fatty acid were identified. Using the same methodology, we also identified many of the same species of glycerophospholipids in resident peritoneal macrophages (RPM), foam cells and murine bone marrow derived macrophages (BMDM).

9. Capture and release of alkyne-derivatized glycerophospholipids using cobalt chemistry

Stephen B. Milne, Keri A. Tallman, Remigiusz Serwa, Carol A. Rouzer, Michelle D. Armstrong, Lawrence J. Marnett, Charles M. Lukehart, Ned A. Porter, H. Alex Brown

Departments of Pharmacology, Biochemistry, Chemistry, and Vanderbilt Institute for Chemical Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Alkyne-modified phospholipids can be unambiguously identified and differentiated from native species in complex mixtures by formation of dicobalthexacarbonyl complexes. This reaction is specific for alkynes and is unaffected by other glycerophospholipid-related moieties. Enrichment of cells with alkyne-derivatized fatty acids or glycerophospholipids followed by solid-phase sequestration and release is a promising new method for unequivocally monitoring individual glycerophospholipids following incorporation into cells. This technique also facilitates lipidomic analysis of substrates and products.

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10. Development of high-throughput platforms for the study of drug-membrane interactions

Claire Stanley[1], Duncan Casey[1], Antony Gee[2], Andrew deMello[1], Oscar Ces[1]

[1]Chemical Biology Centre and Department of Chemistry, Imperial College London, South Kensington Campus, London SW7 2AZ, UK. [2]GSK Clinical Imaging Centre, Imperial College London, Hammersmith Hospital, London W12 0NN, UK.

Understanding how drug molecules interact with our body and what effects they may induce as a result is of fundamental concern to the pharmaceutical industry. Crucially we want to use this knowledge to our advantage during the drug discovery process in order to manipulate a drug's efficacy in vivo - therefore the development of new platforms able to screen numerous desirable drug-membrane interactions and consequently analyse lipid species is of key importance.

We have observed that cationic amphilphilic drugs (CADs), one of the largest groups of drug molecules in the pharmaceutical industry, chemically degrade model lipid membranes to form their corresponding lyso lipids and fatty acids. This phenomenon occurs via an acid catalysed hydrolysis of the ester moieties present in the phospholipids and thus presents itself as a potential new mode of drug transport. In order to examine a large selection of CADs, and hence investigate how degradation rates could relate to their in vivo profiles, the development of assays compatible with high-throughput screening technologies is required. Predominantly our research has sought to utilise 'lab-on-a-chip' based platforms, where water-in-oil droplet systems act as individual experimental vessels that can be manipulated in various ways.

11. Subcellular membrane lipidomics in TLR 4-challenged macrophages

Alexander Y. Andreyev[1], Eoin Fahy[2], Ziqiang Guan[3], Samuel Kelly[4], Xiang Li[2], Jeff G. McDonald[5], Stephen Milne[6], David Myers[6], Andrea Ryan[3], Bonne M. Thompson[5], Elaine Wang[4], Yihua Zhao[2], H. Alex Brown[6], Alfred Merrill[4], Christian R.H. Raetz[3], David Russell[5], Shankar Subramaniam[2], Edward A. Dennis[1]

[1]Department of Chemistry and Biochemistry and Pharmacology, and [2]San Diego Supercomputing Center, University of California, San Diego, La Jolla, CA 92093. [3]Department of Biochemistry, Duke University Medical Center, Durham, NC 27710. [4]School of Biology, Georgia Institute of Technology, Atlanta, GA 30332. [5]UT Southwestern Medical Center, Dallas, TX 75390. [6]Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Lipids are crucial for orchestrating biological processes as signaling molecules and as membrane components that modulate protein function. Understanding of the latter function requires detailed knowledge of the lipid composition at a local level. Activation of macrophages is a key event in inflammation, immune responses and phagocytosis. We report here the mapping of the *subcellular* lipidome of RAW 264.7 macrophages in their basal and activated states. Lipidomics analysis of major membrane lipid categories performed on the major subcellular compartments (nuclei, mitochondria, endoplasmic reticulum (ER), plasmalemma and cytoplasm) identified 229 individual/isobaric species, including 163 phospholipids, 48 sphingolipids, 13 sterols and 5 prenols. Major membranes were shown to substantially diverge in their phospholipid profiles. Activation of macrophages by lipopolysaccharide Kdo_2-Lipid A caused significant changes in the levels of certain phospholipid, sphingolipid and sterol species. Some of these changes occurred globally in all compartments (e.g., increase in the levels of ceramides and cholesterol precursors desmosterol and lanosterol). Others manifested themselves locally, in specific organelles. For example, in mitochondria, oxidized sterols increased whereas highly unsaturated cardiolipins decreased; in ER, highly unsaturated ether-linked phosphatidylethanolamine decreased. We speculate that these results reflect mitochondrial oxidative stress and specific release of arachidonic acid in response to cell activation, respectively.

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12. Effects of omega-3 fatty acids on lipid metabolism and signaling using lipidomic analyses

Joshua D. Brooks, Paul C. Norris, Edward A. Dennis

Departments of Pharmacology and Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093.

While arachidonic acid (an omega-6 fatty acid) and its metabolites play a role in many inflammatory diseases, the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) possess potent anti-inflammatory properties and exert therapeutic benefits. Increased omega-3 fatty acid intake has been shown to alter the balance of omega-6-derived eicosanoid production, but research to date has focused on only a few select lipid metabolites rather than on changes in the cellular-wide eicosadome. Herein we report overall effects of EPA and DHA on the cellular lipidome and the inflammatory eicosadome occurring in RAW264.7 macrophages using novel mass spectrometry techniques (*JBC*, 282, 22834). Our studies have demonstrated that while small variations are seen in the quantity of omega-6 fatty acids found in the cell, major decreases are seen in some (but not all) of the known omega-6 fatty acid-derived eicosanoids forming in macrophages along with a concomitant increase in a few specific omega-3 fatty acid metabolites. Thus, for the first time, the overall variation in cellular lipid metabolites caused by the presence of EPA and DHA has been examined. Taken together, these studies will help to elucidate the likely signaling molecules responsible for and the molecular mechanisms underlying the beneficial effects of EPA and DHA in inflammation. *This work was supported by NIH Grant GM069338.*

13. Lipidomic analysis of carrageenan-induced hyperalgesia reveals essential eicosanoid production in the spinal cord

Darren S. Dumlao[1], Matthew W. Buczynski[1], Camilla I. Svensson[2], Bethany Fitzsimmons[2], Jennifer Wirkus[2], Faith E. Jacobsen[1], Xiao-Ying Hua[2], Tony L. Yaksh[2], Edward A. Dennis[1]

[1]Department of Pharmacology and Department of Chemistry and Biochemistry, and [2]Department of Anesthesiology, University of California San Diego, La Jolla, CA 92093.

Using a high throughput mass spectrometric lipidomics approach, we determined the contribution of bioactive lipids to carrageenan-induced hyperalgesia. Quantitative analysis of CSF and spinal cord tissue for 171 different eicosanoids, ethanolamides and fatty acids revealed 102 distinct lipid species. There was a simultaneous increase of 12-lipoxygenase (12-LOX) and cyclooxygenase (COX) metabolites at 4 hours in CSF that corresponded to the peak of hyperalgesia. In contrast, changes occurred in a temporally disparate manner in the spinal cord, with 12-LOX-derived hepoxilins followed by COX-derived PGE_2, and subsequently the ethanoamide anandamide between 8 and 24 hours in spinal cord. Additionally, spinal bioactive lipids increased bi-laterally, while hyperalgesia was measured to only occur uni-laterally. Systemic treatment with the mu-opioid agonist morphine, the COX inhibitor ketorolac, or the LOX inhibitor NDGA were used to investigate the correlation between spinal bioactive lipid levels and hyperalgesia. The inhibitors reduced allodynia but affected lipid profiles differentially. Morphine did not alter the lipid profile, while ketorolac and NDGA caused a global reduction of many bioactive lipid mediators. These findings suggest that COX and 12-LOX play an important role in the induction of carrageenan mediated hyperalgesia potentially via cross talk between their pathways. Preliminary lipidomic analyses of primary rat glial cell cultures have been conducted to determine the cellular origin of these bioactive lipids. *This work was supported by GM64611 and GM069338 (E.A.D.).*

14. Mitochondrial phosphatase PTPMT1 dephosphorylates phosphatidylglycerol phosphate and is essential for cardiolipin biosynthesis

Ji Zhang[1], Ziqiang Guan[5], Anne N. Murphy[1], Sandra E. Wiley[1] Carolyn Worby[1], James L. Engel[1], Philip Heacock[6], Oanh Kim Nguyen[1], Jonathan Wang[1], Christian R.H. Raetz[5], William Dowhan[6], Jack E. Dixon[1,2,3,4]

[1]Department of Pharmacology, [2]Department of Cellular and Molecular Medicine, and [3]Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093, USA. [4]Howard Hughes Medical Institute, Chevy Chase, MD 20815-6789, USA. [5]Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, USA. [6]Department of Biochemistry and Molecular Biology, University of Texas-Houston Medical School, Houston, TX 77030, USA.

Mitochondria are distinct organelles present in most eukaryotic cells and provide the power source for cellular activities. Dysregulation of mitochondria is associated with a number of pathological conditions, such as neurodegenerative diseases and diabetes. Cardiolipin, a phospholipid that is almost exclusively localized to the inner membrane of mitochondria, plays an essential role in optimizing mitochondrial function. The biosynthetic pathway of cardiolipin has been extensively studied; however, the molecular identity of eukaryotic phosphatidylglycerophosphate (PGP) phosphatase, the enzyme that catalyzes the conversion of PGP to phosphatidylglycerol (PG), remains unknown.

Our laboratory previously discovered the first dual specificity phosphatase targeted to the inner membrane of the mitochondrion, PTPMT1. Knockdown of PTPMT1 has a profound effect on ATP production and potentiates glucose-stimulated insulin secretion. Although initially characterized as a lipid phosphatase with activity towards phosphatidylinositol 5-phosphate (PI(5)P) *in vitro*, our efforts failed to demonstrate that PI(5)P was a biologically important substrate. To study the function of PTPMT1 in a physiologically relevant context, we genetically engineered mice in which the *Ptpmt1* gene has been ablated through homologous recombination. Interestingly, whole body deletion of *Ptpmt1* leads to death of the early embryo, suggesting an essential role for PTPMT1 during mouse development. Subsequently, mouse embryonic fibroblasts with either flox or knockout *Ptpmt1* alleles were derived and an increase in PGP content in the *Ptpmt1*-deficient cells was observed along with a concomitant decrease in PG content by quantitative lipidomic analysis. Furthermore, recombinant PTPMT1 specifically dephosphorylated PGP *in vitro*, indicating that PTPMT1 functions as the PGP phosphatase in eukaryotes. Consistent with its function in cardiolipin biosynthesis, loss of PTPMT1 led to dramatic diminution of cardiolipin production, inhibition of mitochondrial respiration, and fragmentation of mitochondria. Together, our study has uncovered the molecular identity of the eukaryotic PGP phosphatase and identified PTPMT1 as a novel regulator of cardiolipin biosynthesis.

This work was supported by grants from the National Institutes of Health. (DK18024 and NIDDK DK18849 to J.E.D., DK054441 to A.N.M), and the Larry L. Hillblom Foundation (2007-D-016-FEL) to J.Z.

15. Prostaglandin D2 enhances interleukin -1beta- induced cyclooxygenase-2 expression in osteoarthritic cartilage POSTER NOT PRESENTED

Nadia Zayed, Nadir Chabane, Fatima Ezzahra El Mansouri, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Hassan Fahmi

Osteoarthritis Research Unit, Research Centre of the University of Montreal Hospital Center (CR-CHUM), Notre-Dame Hospital, and Department of Medicine, University of Montreal, Montreal, Quebec H2L 4M1 Canada.

Objective: To investigate the effects of prostaglandin D2 (PGD2) on interleukin-1beta (IL-1beta)-induced cyclooxygenase (COX)-2 expression in human cartilage and the signalling pathways involved in these effects.

Methods: chondrocytes were stimulated with IL-1 in the presence or absence of PGD2, and expression of COX-2 protein was evaluated by western-blotting. Messenger RNA (mRNA) expression was analyzed by real-time reverse transcription-polymerase chain reaction. The role of the PGD2 receptors D prostanoid receptor 1 (DP1) and chemoattractant receptor-like molecule expressed on Th2 cells (CRTH2) was evaluated using specific agonists.

Results: PGD2 increased in a dose-dependent manner IL-1-induced COX-2 protein and mRNA expression. DP1 and CRTH2 were expressed and functional in chondrocytes. The effect of PGD2 was mimicked by DK-PGD2 and Indomethacin, selective agonists of CRTH2, but not by BW245C, a selective agonist of DP1. Furthermore, treatment with an anti-CRTH2 antibody reversed the effect of PGD2, indicating that the stimulatory effect of PGD2 is mediated by CRTH2. Activation of CRTH2 is consistent with the activation of a receptor coupled to a phosphoinositide-specific phospholipase, suggesting that the effect of PGD2 is mediated by the CRTH2/PIP2/PKC.

Conclusion: PGD2 stimulates IL-1-induced production of COX-2 by chondrocytes through the CRTH2/PIP2/PKC signalling pathway.

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16. Valproic acid suppresses interleukin-1β-induced microsomal prostaglandin E2 Synthase-1 expression in chondrocytes POSTER NOT PRESENTED

Nadia Zayed, Nadir Chabane, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Hassan Fahmi

Osteoarthritis Research Unit, Research Centre of the University of Montreal Hospital Center (CR-CHUM), Notre-Dame Hospital, and Department of Medicine, University of Montreal, Montreal, Quebec H2L 4M1 Canada.

Introduction: Microsomal prostaglandin E2 Synthase (mPGES)-1 catalyzes the terminal step in the biosynthesis of PGE_2. Early growth response factor-1 (Egr-1) is a key transcription factor in the regulation of mPGES-1. In the present study we examined the effects of valproic acid (VA), a histone deacetylase (HDAC) inhibitor, on interleukin (IL)-1β-induced mPGES-1-expression in human chondrocytes.

Methods: Chondrocytes were stimulated with IL-1 in the absence or presence of VA, and the level of mPGES-1 protein and mRNA expression were evaluated using Western blotting and real-time reverse-transcription polymerase chain reaction, respectively. The mPGES-1 promoter activity was analyzed in transient transfection experiments. Egr-1 recruitment to the mPGES-1 promoter were evaluated using chromatin immunoprecipitation (ChIP) assays.

Results: VA dose-dependently suppressed IL-1β-induced mPGES-1 protein and mRNA expression as well as its promoter activation. Treatment with VA did not alter IL-1-induced Egr-1 expression, nor its recruitment to the mPGES-1 promoter, but prevented its transcriptional activity.

Conclusion: Our study demonstrates that VA inhibits IL-1-induced mPGES-1 expression in chondrocytes. The suppressive effect of VA was not due to reduced expression or recruitment of Egr-1 to the mPGES-1 promoter.

17. Interleukin-1-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in human OA chondrocytes is associated with histone H3K4 methylation

POSTER NOT PRESENTED

Fatima Ezzahra El Mansouri, Nadia Zayed, Nadir Chabane, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Hassan Fahmi

Osteoarthritis Research Unit, University of Montreal Hospital Research Center (CRCHUM), Notre-Dame Hospital, and Department of Medicine, University of Montreal, Montreal, Quebec H2L 4M1, Canada.

Objective: Increased expression of inducible NO synthase (iNOS) and cyclooxygenase (COX)-2 plays a key role in the pathogenesis of osteoarthritis (OA). Methylation of lysine 4 on histone H3 (H3K4) was shown to be of fundamental importance in the regulation of gene expression. In the present study, we investigated the role of H3K4 methylation in interleukin-1β (IL-1)-induced COX-2 and iNOS expression in human OA chondrocytes.

Methods: Chondrocytes were stimulated with IL-1 for various time periods and the expression of iNOS and COX-2 mRNAs and proteins were evaluated using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting, respectively. H3K4 methylation at the iNOS and COX-2 promoters was evaluated using chromatin immunoprecipitation (ChIP) assays. The role of histone methylation was further evaluated using the methyltransferase inhibitor, 5'-deoxy-5'(methylthio) adenosine (MTA).

Results: IL-1 induced iNOS and COX-2 mRNA and protein in a dose- and time-dependent manner. The induction of iNOS and COX-2 expression by IL-1 was associated with H3K4 di- and trimethylation at the iNOS and COX-2 promoters, whereas the levels of H3K4 monomethylation remained unchanged. Treatment with MTA inhibited IL-1-induced H3K4 methylation as well as IL-1-induced iNOS and COX-2 expression.

Conclusion: These results indicate that H3K4 methylation contributes to IL-1-induced iNOS and COX-2 expression and suggest that this pathway could be a potential target for pharmacological intervention in the treatment of OA.

18. Myeloid differentiation primary response protein 88 couples reverse cholesterol transport to inflammation*

Kathleen A. Smoak[1], Jim J. Aloor[1], Jennifer Madenspacher[1], B. Alex Merrick[1], Jennifer B. Collins[2], Xuewei Zhu[3], Giorgio Cavigiolio[4], Michael N. Oda[4], John S. Parks[3], Michael B. Fessler[1]

[1]Laboratory of Respiratory Biology and [2]Microarray Core Facility, National Institute for Environmental Health Sciences, Research Triangle Park, NC 27709 USA. [3]Department of Pathology/Section on Lipid Sciences, Wake Forest University Health Sciences, Winston-Salem, NC 27157 USA. [4]Children's Hospital Oakland Research Institute, Oakland, CA 94609 USA.

Crosstalk exists in mammalian cells between cholesterol trafficking and innate immunity signaling to microbes. Apolipoprotein A-I (apoA-I), a serum apolipoprotein that induces anti-atherogenic efflux of macrophage cholesterol, is widely described as anti-inflammatory because it neutralizes bacterial lipopolysaccharide (LPS). Conversely, LPS-induced inflammation is pro-atherogenic. However, whether innate immunity plays an endogenous, physiological role in host cholesterol homeostasis in the absence of infection is undetermined. We report that apoA-I signals in the macrophage through CD14, Toll like Receptor (TLR)2, and TLR4, utilizing Myeloid Differentiation Primary Response Protein 88 (MyD88)-dependent and –independent adaptor pathways, to activate MAPKs and nuclear factor-κB. ApoA-I had no detectable LPS contamination, and its effects were unaffected by polymyxin B and ablated by protease treatment. Further, while apoA-I induction of TNFα was unchanged in ATP Binding Cassette Transporter A1 (ABCA1) null macrophages, LPS and PAM3CSK4 induction of TNFα was increased in these cells. Whereas apoA-I has canonically been considered anti-inflammatory, we find that it induces multiple NF-κB and MyD88-dependent cytokines in the macrophage, including some reported to modulate cholesterol efflux. Neutrophils were recruited to the peritoneum of apoA-I injected mice, indicating acute inflammation; however, there was significantly less recruitment in mice null for MyD88 or TLR4. Moreover, we find that MyD88 plays a critical role in cholesterol efflux from murine and human macrophages *in vitro*. In the mouse, disposal of cellular cholesterol into the plasma and from there to the feces is deficient from transplanted MyD88 null macrophages, indicating that MyD88 is critical for reverse cholesterol transport *in vivo*. Taken together, this work identifies apoA-I as a novel endogenous stimulus of innate immunity that couples cholesterol trafficking to inflammation through MyD88, and identifies innate immunity as a physiologic signal in cholesterol homeostasis.

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19. The different gene profiles by oxysterols in macrophage

Norihito Shibata[1], Bonne M. Thompson[2], Ruth Yu[3], Ronald M. Evans[3], David W. Russell[2], Christopher K. Glass[1]

[1]Dept. of Cellular & Molecular Medicine, University of California, San Diego, La Jolla, CA. [2]Dept. of Molecular Genetics, UT Southwestern Medical Ctr, Dallas, TX. [3]Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, CA.

Oxysterols are oxygenated derivatives of cholesterol. Endogenous oxysterols are thought to be produced in proportion to intracellular cholesterol levels or be formed during biosynthesis of bile acids, steroid hormones, and oxidation of low-density lipoprotein. In vivo, oxysterols act as intermediates in cholesterol catabolism, regulators of lipid metabolism, suppressors of inflammation, and as toxic factors for proatherogenic effect. Macrophages play a central role in the atherogenic process as modulators of both lipid metabolism and immune responses. Recently, LIPID MAPS consortium revealed that the activation of Toll-like receptor 4 (TLR4) induces the production of oxysterols, such as 25-hydroxycholesterol (25OHC), and the expression of biosynthetic enzymes for oxysterols, such as cholesterol 25 hydroxylase, that converts cholesterol to 25OHC. But the physiological meaning of this induction of oxysterols remains unclear. To evaluate the roles of oxysterols induced by the activation of TLR4, gene expression profiling experiments were performed using bone marrow-derived macrophages (BMDMs) treated with 25OHC 24(S), 25 epoxycholesterol (EC), GW3965 (a synthetic agonist for liver X receptor (LXR)), or Kdo2 lipidA (an activator for TLR4). As expected, EC induced the genes involved in lipid metabolism, including established LXR target genes such as *Abcg1* and *Apoc1*. The profiles for EC was quite similar to those for GW3965. However, unexpectedly, the gene profiles for 25OHC was quite different from those for EC and GW3965, but overlapped to a greater extent with the profile for Kdo2 lipid A. These results suggest that, among oxysterols, 25OHC may have a unique function in macrophage during inflammation.

20. A novel role of phospholipids in brain prostanoid level regulation

Colin K. Combs, Eric J. Murphy, Mikhail Y. Golovko

Department of Pharmacology, Physiololgy, and Therapeutics, University of North Dakota, Grand Forks, ND.

Despite the broad involvement of prostanoids (PG) in normal and pathophysiological processes in the nervous system, the mechanism that regulates brain PG levels is poorly understood. To address a mechanism for a rapid (within seconds) increase in brain PG levels upon stimulation, the levels of free and esterified onto phospholipids PG were measured using LC-MS/MS analysis that allows for PG separation from all tested isoprostanes. Mouse brains were fixed using head focused microwave irradiation to heat denature proteins involved in PG metabolism. PG levels were measured in control mice, or upon treatment with LPS, global brain ischemia, or indomethacin. A significant pool of PG was found esterified onto phospholipids at basal levels that was released with PLA_2. The esterified PG pool was 27-fold greater than the levels of free prostanoids found in brain, demonstrating a large, PLA_2 releasable pool of prostanoids. These esterified PG were rapidly released in vivo by 30 sec of global ischemia or upon LPS treatment, and contributed to the 30-fold increase in free prostanoid levels under these conditions. In addition, after 20 min of THP-1 cell incubation with exogenously added deuterated PGD_2, 43% of total PGD_2 found in phospholipids were labeled with PGD_2-d_4. Importantly, esterified PG pool was decreased upon treatment with indomethacin, indicating COX dependant pathway for its synthesis. These results led us to hypothesize a novel pathway accounting for the rapid increase in brain PG mass via the rapid (<1 min) mobilization of a releasable pool of preformed PG esterified onto brain phospholipids. *Supported by NIH Grant 1R21NS064480 - 01A1 (MYG), ND EPSCoR grant EPS-0814442 (MYG), UND New Faculty Scholar Award (MYG), and by NIH funded COBRE Mass Spec Core Facility Grant 5P20RR017699.*

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21. Lipid profiling of tumor lipid droplets by UPLC/TOF MS

Hayley Crowe[5], John P. Shockcor[1], Ladan Mirbahai[2], Martin Wilson[3], Christopher S. Shaw[2], Carmel McConville[3], Roger D. G. Malcomson[3], Risto A. Kauppinen4, Andrew C. Peet[3], Julian L. Griffin[1]

[1]Department of Biochemistry, University of Cambridge, Cambridge, CB3 0LT UK. [2]School of Sport and Exercise Sciences & School of Cancer Sciences, University of Birmingham, Birmingham, B4 6NH UK. [3]Dept of Histopathology, Birmingham Children's Hospital NHS Foundation Trust, Birmingham, B4 6NH UK. [4]Dartmouth Medical School, Hanover, NH 03755, USA. [5]Waters corporation, Milford, MA 01757, USA.

It was previously observed that during cell death in tumors there is a characteristic increase in lipid species. During apoptosis in glioma, these lipid species are highly enriched for polyunsaturated fatty acids and occur in droplets within the cytoplasm. In addition to phosphatidylcholines these droplets also contain triglycerides in intact tumours. However, the source of these lipids and their exact role within the cell remain a mystery.

Here we have employed an oaTof ion-mobility mass spectrometer coupled to an UPLC to characterize the content of these lipid droplets. Glioma cells treated with thymidine kinase were injected into the brain of rats. Apoptosis was induced by treatment with gancyclovir. Lipids were extracted from the tumors using 2:1 chloroform/methanol v/v. The lipids were separated on a UPLC system with UPLC HSS T3 (2.1x100 mm, 1.7uM, 65°C) column. The flow rate was 0.5mL/min. Mobile phase A was 2:3 acetonitrile/water, 10mM ammonium acetate pH 5.0, and mobile phase B was 1:9 acetonitrile/isopropanol with 10mM ammonium acetate. Experiments were carried out for both ESI+ and ESI- , acquiring data over a range of m/z 150 to 1200. The mass spectrometer was collecting data in two separate functions simultaneously called MS^E. The first function used low collision energy so that precursor ion data was obtained for the lipid species; the second function used elevated collision energy so that production data was obtained. Related parent and product ion information is chromatographically time-aligned in a post acquisition processing step. We were able to identify polar lipid species and triglycerides in the tumor lipid droplets present during apoptosis. The exact mass information obtained provides a more comprehensive profiling of the lipid species in the droplets and should aid in our understanding of how these lipid droplets are produced, and whether they may form a diagnostic signal for cell death in tumours.

22. Heat Shock Protein 70B' (HSP70B') expression and release in response to human oxidized LDL immune complex in macrophages

Kent J. Smith[1], Waleed O. Twal[1], Farzan Soodavar[1], Gabriel Virella[2], Maria F. Lopes-Virella[3,4], Samar M. Hammad[1]

[1]Department of Regenerative Medicine and Cell Biology, [2]Microbiology and Immunology, and [3]Division of Endocrinology, Department of Medicine, Medical University of South Carolina, Charleston, SC 29425. [4]Department of Veteran Affairs, Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC 29401.

Heat shock proteins (HSPs) have been implicated in the activation and survival of macrophages. This study examined the role of HSP70B', a poorly characterized member of the HSP70 family, in response to oxidatively modified LDL (oxLDL) and immune complexes prepared with human oxLDL and purified human antibodies to oxLDL (oxLDL-IC) in monocytic and macrophage cell lines. Immunoblot analysis of cell lysates and conditioned medium from U937 cells treated with oxLDL alone revealed an increase in intracellular HSP70B' protein levels accompanied by a concomitant increase in HSP70B' extracellular levels. Fluorescence immunohistochemistry and confocal microscopy however demonstrated that oxLDL-IC stimulated the release of HSP70B', which co-localized with cell-associated oxLDL-IC. In HSP70B'-GFP-transfected mouse RAW 264.7 cells, oxLDL-IC-induced HSP70B' co-localized with membrane-associated oxLDL-IC as well as lipid moiety of internalized oxLDL-IC. Furthermore, the data demonstrated that HSP70B' is involved in cell survival, and this effect could be mediated by sphingosine kinase 1 (SK1) activation. An examination of regularly implicated cytokines revealed a significant relationship between HSP70B' and the release of the anti-inflammatory cytokine interleukin-10 (IL-10). siRNA knockdown of HSP70B' resulted in a corresponding decrease in SK1 mRNA levels and SK1 phosphorylation, as well as increased release of IL-10. In conclusion, these findings suggest that oxLDL-IC induce the synthesis and release of HSP70B', and once stimulated, HSP70B' binds to cell-associated and internalized lipid moiety of oxLDL-IC. The data also implicate HSP70B' in key cellular functions such as regulation of SK1 activity, and release of IL-10; which influence macrophage activation and survival.

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23. Embryonic expression of leukotriene biosynthetic proteins

Tobias Strid, Hong Qian, Cecilia Karlsson, Mats Söderström, Mikael Sigvardsson, Sven Hammarström

Department of Clinical and Experimental Medicine, Division of Cell Biology, Linköping University, SE-581 85 Linköping, Sweden.

Leukotrienes (LT) are potent pro-inflammatory mediators formed from arachidonic acid. At least four proteins are required for LT biosynthesis viz. 5-lipoxygenase (5LO), 5LO- activating protein (FLAP), leukotriene A_4 hydrolase, and leukotriene C_4 synthase. LT have important roles in innate immunity and exert their effects through G-protein coupled receptors. They have key functions in inflammatory disorders such as asthma, allergy, atherosclerosis, myocardial infarction and stroke and have been suggested to be involved in fetal brain development. We have investigated the expression of leukotriene biosynthetic enzymes during mouse embryonic development. Data obtained by *in situ* hybridisation, immunohistochemistry and qRT-PCR showed that these proteins are expressed in fetal liver from e11.5 until birth. The cellular origin of the expression was determined after sorting of different cell populations from e15.5 fetal liver using a fluorescence-activated cell sorter (FACS) as hematopoietic cells colonize the liver from around e10 and liver is the main hematopoietic organ during embryonic development. Little or no expression was detected in hepatocytes and other non-hematopoietic cells in the fetal liver. Expression was also largely absent from adult liver but high in adult bone marrow hematopoietic cell compartments. Sorted hematopoietic cell populations from fetal liver and adult bone marrow showed high expression of LT biosynthetic proteins in lineage marker negative (Lin^-), SCA1 and KIT^{high} (LSK) cells and mature myeloid ($MAC1^+GR1^+$) cells whereas intermediate developmental cells, including common myeloid progenitors and common lymphoid progenitors, displayed lower expression. This expression pattern of LT biosynthetic proteins suggests potential novel roles for leukotrienes in early hematopoiesis.

24. Sterol modified lipids-non-exchangeable lipids for stable lipid vesicle formation

Francis C. Szoka, Zhaohua Huang

Bioengineering and Therapeutic Science, University of California San Francisco, CA 94143-0912.

We synthesized a family of sterol-modified glycerophospholipids (SML) in which the sn-1 or sn-2 position is covalently attached to cholesterol and the alternative position contains an aliphatic chain. The SML were used to explore how anchoring cholesterol to a phospholipid affects cholesterol behavior in a bilayer. SMLs formed liposomes upon hydration and when they are mixed with C14 to C18 diacylglycerophospholipids, the thermotropic phase transition, measured by differential scanning calorimetry, is eliminated at the SML equivalent of 25 to 40 mole percent free cholesterol. The effects of the SML on the transition temperature and enthalpy of the transition of synthetic PC are similar to the effects of free cholesterol. Osmotic-induced contents leakage from SML (C14) liposomes is the same as in dimyristoylphosphatidylcholine/ cholesterol: 1/1 (mole ratio) liposomes. However SML liposomes are exceptionally resistant to contents release in serum at 37ºC. The exchange rate of an SML between bilayers is greater than 100 fold less than the exchange rate of free cholesterol. SMLs stabilize bilayers but do not exchange, so provide a new tool for biophysical studies on membranes and membrane proteins. *Funded by NIH GM061851.*

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25. Saturated fatty acid lauric acid activates Toll-like receptor 4 by inducing dimerization and recruitment of the receptor into lipid rafts in a ROS dependent manner

Scott W. Wong[1], Myung-Ja Kwon[1], Ryan Snodglass[1], Augustine M.K. Choi[2], Hong-Pyo Kim[2], Kiichi Nakahira[2], Daniel Hwang[1]

[1]Western Human Nutrition Research Center/ARS/USDA and Department of Nutrition, University of California Davis, CA 95616. [2]Division of Pulmonary and Critical Care Medicine, Harvard Medical School, Boston, MA 02115.

Results from our previous studies demonstrated that saturated and polyunsaturated fatty acids reciprocally modulate the activation of TLR4. However, the underlying mechanism has not been understood. There is no evidence that saturated fatty acids directly bind TLR4. The saturated fatty acid lauric acid (C12) induces dimerization and recruitment of TLR4 into lipid rafts, and enhanced the association of TLR4 with MD-2 and downstream adaptor molecules leading to target gene expression. However, docosahexaenoic acid (DHA) inhibited LPS- or C12-induced dimerization and recruitment of TLR4 into lipid rafts. Dimerization of TLR4 induced by C12 was accompanied by activation of NADPH oxidase (NOX) and reactive oxygen species (ROS) generation. An inhibitor of NOX suppressed C12-induced dimerization of TLR4. Together, these results demonstrate that C12 and DHA reciprocally modulate TLR4 activation by regulation of the dimerization and recruitment of TLR4 into lipid rafts, which are coupled events mediated at least in part by NOX-dependent ROS generation. These results provide a new insight in understanding the mechanism by which fatty acids differentially modulate TLR4-mediated signaling pathway and consequent inflammatory responses which are implicated in the development and progression of many chronic diseases (*supported by grants: DK064007, NIH, 2001-35200-10721,USDA and funds from the Western Human Nutrition Research Center/ARS/USDA*).

26. Functional analysis of lysoPI acyltransferase 1 (LPIAT1)

Hiroyuki Arai, Takao Inoue

Graduate School of Pharmaceutical Sciences, University of Tokyo, and CREST JST, Japan.

Phosphatidylinositol (PI), a component of membrane phospholipids, serves as the precursor of important signaling molecules such as phosphoinositides. PI is unique in its fatty acid composition, i.e. 1-stearoyl-2-arachidonoyl (18:0/20:4) PI is the major molecular species in mammals. The molecular species is thought to be formed in a fatty acid remodeling reaction after the *de novo* synthesis of PI; however, the physiological significance of the fatty acid remodeling of PI remains to be elucidated.

In an RNAi-based genetic screen using *C. elegans*, we have recently identified an evolutionarily acyltransferase, named *mboa-7*/LPIAT1, that selectively incorporates arachidonic acid at the *sn*-2 position of PI (Lee *et al.*, *Mol Biol Cell*, 2008). The LPIAT1 KO mice lack arachidonoyl-CoA:LPI acyltransferase activity and show growth defects, suggesting that the fatty acid remodeling of PI is important for development in mammals. In this meeting, we will present our recent data on LPIAT1 KO mice. We will also talk about identification of enzymes that are involved in the fatty acid remodeling at the *sn*-1 fatty acid of PI.

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27. Identification of key residues determining species differences in inhibitor binding of Microsomal Prostaglandin E Synthase 1

Sven-Christian Pawelzik[1], Narasimha Rao Uda[1], Linda Spahiu[3], Caroline Jegerschöld[2], Patric Stenberg[3], Hans Hebert[2], Ralf Morgenstern[4], Per-Johan Jakobsson[1]

[1]Department of Medicine, Rheumatology Unit, Karolinska Institutet, S-171 77 Stockholm, Sweden. [2]Department of Biosciences and Nutrition, Karolinska Institutet and School of Technology and Health, Royal Institute of Technology, Novum, S-141 57 Huddinge, Sweden. [3]Actar AB, Nobels väg 3, S-171 77 Solna, Sweden and NovaSAID AB, Fogdevreten 2a, S-171 77 Solna, Sweden [4]Institute of Environmental Medicine, Karolinska Instiutet, S-171 77 Stockholm, Sweden.

Microsomal Prostaglandin E Synthase-1 (MPGES1) is induced during an inflammatory reaction from low basal levels by pro-inflammatory cytokines and subsequently involved in the production of the important mediator of inflammation, prostaglandin (PG) E_2. Non-steroidal anti-inflammatory drugs (NSAIDs) prevent PGE_2 production by inhibiting the upstream enzymes Cyclooxygenase (COX)-1 and COX-2. In contrast to these conventional drugs, a new generation of NSAIDs targets the terminal enzyme MPGES1. Some of these compounds potently inhibit human MPGES1 but do not have an effect on the rat orthologue. We investigated this interspecies difference in a rat/human chimeric form of the enzyme as well as in several mutants and identified key residues Thr-131, Leu-135, and Ala-138 in human MPGES1 that play a crucial role as gate keepers for the active site of MPGES1. These residues are situated in transmembrane helix (TM) 4, lining the entrance to the cleft between two subunits in the protein trimer, and regulate access of the inhibitor in the rat enzyme. Exchange towards the human residues in rat MPGES1 was accompanied with gain of inhibitor activity, while exchange in human MPGES1 towards the residues found in rat abrogated inhibitor activity.

Our data give evidence for the location of the active site at the interface between subunits in the homotrimeric enzyme and suggest a model of how the natural substrate PGH_2, or competitive inhibitors of MPGES1, enter the active site via the phospholipid bilayer of the membrane.

28. Investigating neurodegenerative disorder systems using USPIO-nanoparticles with (SECARS) and stimulated raman scattering microscopy **POSTER NOT PRESENTED**

Lina H. Machtoub

Universitätsklinik für Radiodiagnostik, Innsbruck Medical University, Innsbruck, Austria.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease, pathologically characterized by loss of motor neurons. Recently intensive efforts have been made on investigating the mechanism underlying motor neuron degeneration which up to now remains incompletely understood. In this study, we investigated a novel application of Surface-Enhanced Coherent Anti-Stokes Raman Scattering (SECARS) and Stimulated Raman Scattering (SRS) microscopy for imaging intravenously injected ultra small paramagnetic iron oxide (USPIO) nanoparticles in transgenic amyotrophic lateral sclerosis experimental model. Experiments were performed on transgenic amyotrophic lateral sclerosis rat model, expressing multiple copies of mutated (G93A) human SOD-1 gene (hSOD-1^{G93A}), magnetically labelled with antibodies tagged with ultra small paramagnetic iron oxide nanoparticles (USPIO). Brain tissues, taken from ALS and wild type (WT) rat models, were investigated by *ex vivo* SECARS, SRS and immunohistochemistry. Marked intensity enhancements have been observed in specific pathological regions of the ALS brain. The results obtained correlated SECARS enhancements to selective association of lipids to up-taken USPIO that shows high distribution in regions with abnormal accumulation of lipids mostly observed in the brainstem and midbrain regions. The results presented are compared with our previous studies on SPIO-injected animals which showed specific selective association of lipids in cells of the reticuloendothelial system to uptaken SPIO.The results presented show the promising potential of SECARS and Stimulated Raman Scattering microscopy in investigating neurodegenerative disorders in experimental systems using USPIO particles as molecular nanoprobes.

Acknowledgement: Thanks to Prof. P. R. Andjus and D. Bataveljic, University of Belgrade, for providing the ALS model.

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29. Stability and analysis of eicosanoids and docosanoids in culture media

Krishna Rao Maddipati

Department of Pathology, Wayne State University, Detroit, MI 48202.

Metabolism of polyunsaturated fatty acids such as arachidonic acid and docosahexaenoic acid lead to highly physiologically active compounds, generally termed as eicosanoids and docosanoids, respectively. Given the importance of these bioactive lipid mediators in health and disease, these compounds are extensively used in both *in vitro* and *in vivo* experimentation. While eicosanoids such as prostaglandin $F_{2\alpha}$, prostacyclin, prostaglandin E_2, etc., are used clinically and their stability in aqueous solutions as well as their biological stability was studied in detail, stability of a majority of eicosanoids and docosanoids in aqueous solutions and in standard tissue culture conditions was not examined systematically. The present study evaluated the stability of eicosanoids and docosanoids in tissue culture media with respect to incubation time, temperature, and composition of the media using multiple reaction monitoring (MRM) LC-MS. Stability of the organic solvent-free aqueous stock solutions of eicosanoids and docosanoids were also evaluated with respect to time and temperature of storage. Stability of the precursor fatty acids in culture media and contribution of the eicosanoids and docosanoids derived from autoxidation fatty acids as well as from sera used for tissue culture were analyzed to assess their influence on the quantitative aspects of experimentation. The results provide a comprehensive and valuable data set on the stability of these bioactive lipid mediators to guide *in vitro* experimentation.

30. Metabolomic and genomic analysis of cafeteria-diet induced macrophage infiltration of adipose tissue reveals a robust model of obesity, inflammation and glucose intolerance

Brante P. Sampey[1], Matt Wilkerson[2], Amanda M. Vanhoose[3], Helena Winfield[4], Olga Ilkayeva[4], Michael J. Muehlbauer[4], Deborah M. Muoio[4,5,6], Chris B. Newgard[4,5], David Neil Hayes[2], Liza Makowski[1]

[5]Department of Pharmacology, [6]Department of Endocrinology, [4]Stedman Center for Nutrition and Metabolism, Duke University Medical Center, Durham, NC. [3]Department of Molecular Physiology & Biophysics, Vanderbilt University Medical Center. [1]Department of Nutrition, Gillings School of Global Public Health, School of Medicine; University of North Carolina at Chapel Hill. [2]Department of Medicine, Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Macrophages infiltrate adipose tissue at the onset of weight gain and directly contribute to fatty inflammation, insulin resistance, and obesity. Recent reports estimate that children consume up to 25% of calories from high-fat, high-salt snacks. To investigate the obesogenic and inflammatory consequences of a high-fat, high-salt "Cafeteria diet" (CD) consisting of chow plus daily excess of 3 varied human snacks compared to a traditional 45% high fat (HF) diet in rodent models, male Wistar rats were fed HF, CD or appropriate chow controls for 15 weeks. Body weight increased significantly at two weeks and remained elevated on CD, whereas traditional HF-fed rats did not become obese relative to controls. Hyperinsulinemia and glucose intolerance was exaggerated in the CD-fed rats compared to controls and HF-fed rats. Metabolomic profiling of CD-fed rats indicated elevated lipolysis (plasma nonesterified fatty acids, NEFAs) and mitochondrial dysfunction in serum, liver, muscle and adipose. While both diets resulted in altered gene expression in epididymal adipose tissue, CD resulted in greater change in genes associated with increased glucose metabolism and the immune response. Gene Set Expression Analysis (GSEA) revealed regulation of several gene ontology (GO) categories: increases in innate immune response, response to lipopolysaccharide, IKK/NF-KappaB Cascade and decreased insulin receptor signaling. GSEA analysis of the stromovascular fraction of adipose supports elevated inflammation and glycolysis in CD-fed rats. Indeed, histological analysis of the enlarged epididymal adipose tissue identified greater macrophage infiltration in rats fed CD versus HF. In sum, the Cafeteria Diet provided a more potent and robust model of diet-induced obesity compared to traditional HF diet, creating a phenotype of exaggerated obesity with glucose intolerance and inflammation. This model provides a unique platform to study the biochemical and physiological mechanisms of obesity and obesity-related disease states that are pandemic in Western civilization today.

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31. Computational analysis of data from LC ESI-MS/MS of sphingolipids using a peak detection algorithm that eliminates manual peak detection and integration

David Goldberg[1], Samuel Kelly[2], Elaine Wang[2], M. Cameron Sullards[3], Alfred H. Merrill, Jr.[2,3]

[1]Computer Science Laboratory, Palo Alto Research Center, Palo Alto, CA 94304. [2]Schools of Biology, and [3]Chemistry and Biochemistry, Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332-0230.

Liquid chromatography, electrospray ionization-tandem mass spectrometry (LC ESI-MS/MS) has been used to identify and quantitate sphingolipids in complex mixtures such as cells, tissues and other biofluids[1]. Lipidomic analysis of sphingolipids generates an enormous quantity of data from even a single experiment and management of this data is very challenging. One of the time-consuming steps is the need to perform manual peak detection for many of the data sets. This report describes a peak detection algorithm that simplifies data analysis by eliminating manual peak detection and integration. The same approach would be useful for analysis of other categories of compounds.

[1]Shaner RL, Allegood JC, Park H, Wang E, Kelly S, Haynes CA, Sullards MC, Merrill AH Jr. (2009) Quantitative analysis of sphingolipids for lipidomics using triple quadrupole and quadrupole linear ion trap mass spectrometers. *J Lipid Res*, 50:1692-1707.

32. Quantitative analysis of traditional ceramides (N-acyl-sphingoid bases) and 1-deoxy-ceramides (N-acyl-1-deoxy- and -1-desoxymethyl-sphingoid bases) by reverse phase liquid chromatography electrospray ionization tandem mass spectrometry

Sibali Bandyopadhyay[1], Hyejung Park[2], M. Cameron Sullards[1], Alfred H. Merrill, Jr.[1]

[1]Georgia Institute of Technology, Atlanta, GA. [2]Boston University School of Medicine, Boston, MA.

Mammalian sphingolipids have been thought to be derivatives of sphinganine, which is produced after condensation of L-serine and a fatty acyl-CoA by serine palmitoyltransferase (SPT). However, recent studies (1) have found that SPT also accommodates L-alanine and glycine to produce 1-deoxy- and 1-desoxymethyl-sphinganines, respectively, which are N-acylated to 1-deoxy- dihydroceramide and 1-desoxymethyl-dihydroceramides (DHCer). These novel sphingolipids are found in small amounts in cells in culture, animals tissues, and human blood (1); moreover, some are elevated in human sensory neuropathy type 1 (HSN1) (2), an inherited disease due to mutations in SPT. Hence, it is important to establish a method that can distinguish and quantify each of these categories of compounds.

This report describes a method for their quantitative analysis by liquid chromatography electrospray ionization tandem mass spectrometry (LC ESI-MS/MS) in positive ionization mode using an Applied Biosystems 4000 QTrap. After optimization of the LC, ionization and fragmentation conditions with synthetic standards, protocols were established for quantitation of the subspecies found in RAW264.7 cells and human plasma (NIST) using multiple reaction monitoring (MRM). The standard curves showed a linear response between 0.01 to 100 pmol (total on column).

Further analysis of human plasma samples obtained from the Division of Medical Genetics in the Department of Human Genetics at Emory University School of Medicine found statistically significant correlations between plasma serine and dihydroceramides, plasma alanine and 1-deoxydihydroceramides, and plasma glycine and 1-desoxymethyl-dihydroceramides. Therefore, it is likely that the types of sphingoid bases found in humans can be controlled by the amino acid status of the individual, which has provocative implications for the possible biological functions of these compounds and their roles in disease.

Supported the LIPID MAPS grant GM-069338.

1. Zitomer et al., J. Biol. Chem. 284, 4786-4795 (2009).
2. Penno et al., J. Biol. Chem. (In press).

33. Sphingolipidomic analysis of NIST human plasma

Kristin N. Jones, Rebecca L. Shaner, Elaine Wang, Samuel L. Kelly, Sibali Bandyopadhyay, M. Cameron Sullards, Alfred H. Merrill, Jr.

Georgia Institute of Technology, Atlanta, GA 30332.

Sphingolipids are found in all mammalian tissues and blood (1), therefore, the structural diversity in human plasma was explored using lipidomics methods developed by the LIPID MAPS Consortium (2) and a human plasma standard reference material (SRM) generated by the NIDDK in collaboration with the National Institute of Standards (NIST). These analyses found approximately 200 sphingolipids. Sphingomyelins (SM) accounted for the largest fraction (i.e., >100 subspecies totaling 303 nmol/mL, 22.8 mg/dL); the most prevalent sphingoid bases were sphingosine (d18:1, 61%), sphingadiene (d18:2, 18%), sphinganine (d18:0, 9.5%), and d16:0-sphingosine; and the fatty acids ranged in chain length from 13 to 28 carbons, with varying degrees of saturation. Fewer ceramide monohexose (CMH—comprised of glucosyl- and galactosyl-ceramides) species were detected because the amount of CMH was smaller (e.g., 2.3 nmol/mL, 0.180 mg/dL); the most common species were glucosylceramides consisting of the d18:1-ceramide backbone and fatty acyl groups ranging from 14 to 26 carbons, with a higher proportion of very-long-chain Cer than SM. The backbone spectrum of Cer was similar to that of SM and CMH (41 subspecies totaling 12 nmol/mL and 0.75 mg/dL), but the very-long-chain Cer were even higher. Two novel categories of ceramides with 1-deoxy- and 1-(desoxymethyl)-sphingoid base backbones were also identified. Sphingosine 1-phosphate was the major free sphingoid base found in NIST plasma, followed by sphingosine, sphinganine and sphinganine 1-phosphate. In addition to d18:1 sphingosine 1-phosphate, there were smaller amounts of other chain lengths (d16:1 to d19:1). These studies constitute the most in-depth, quantitative analysis of the structural diversity of sphingolipids in human plasma that has thus far been conducted. *Supported by NIH grant GM069338 for the LIPID MAPS Consortium.*

1. Merrill AH Jr et al. (2007) *Trends in Biochem. Sci*. 32: 457-468.
2. Shaner RL et al. (2009) *J. Lipid Res*. 50:1692-1707.

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34. Age-dependent changes in the sphingolipid composition of CD4^{+} T cell membranes and immune synapses

Zhaofeng Huang[1+], Melissa G. Marko[1], Alberto Molano[1], Stephen C. Bunnell[2] ,Dayong Wu[1], Alfred H. Merrill, Jr.[3], Simin Nikbin Meydani[1,2]

[1]Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, [2]Department of Pathology, Sackler Graduate School of Biomedical Sciences, Tufts University, Boston, MA.[3]School of Chemistry and Biochemistry and Biology, The Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia, 30332.

Optimal T cell activation requires the formation of an immunological synapse (IS) and is driven by signaling 'microclusters' that associate with ordered membrane microdomains. Aging is associated with impaired IS formation and T cell activation. We hypothesized that age-dependent changes in T cell lipid profiles impair IS formation and T cell activation in the aged. Total cell membrane (TCM) and IS fractions were prepared from the CD4+ T cells of young and old mice and subjected to 'sphingolipidomic' analysis. Both TCM and IS of aged CD4+ T cells have significantly higher levels of sphingomyelins and ceramides than young T cells. A subset of the lipids containing C14, C18:1, C26, and C26:1 fatty acid (FA) species was preferentially enriched in the IS. Young IS were enriched in C14 ceramide, C18:1 ceramide, C26:1 ceramide, and C14 hexosylceramide. Since sphingolipids are implicated in T cell activation and function, age-dependent changes in the FA chain lengths and sphingolipid species retained in IS could influence T cell activation and function and may contribute to functional T cell defects in the aged.

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[+]Current address: Zhongshan Medical School, Sun Yat-Sen University, 74 Zhongshan Er Road, Guangzhou, Guangdong, 510080 China

35. Oxidized cholesteryl esters activate macrophage via toll-like receptor-4

Soo-Ho Choi[1], Richard Harkewicz[2], Felicidad Almazan[1], Yury I. Miller[1]

[1]Department of Medicine and [2]Departments of Pharmacology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093.

Toll-like receptor-4 (TLR4) recognizes microbial pathogens, such as lipopolysaccharide (LPS), and mediates LPS-induced proinflammatory cytokine secretion, as well as uptake of microbes by macrophages. In addition to exogenous pathogens, TLR4 recognizes modified self, such as minimally oxidized low-density lipoprotein (mmLDL). We found that polyoxygenated cholesteryl ester (CE) hydroperoxides are active components of mmLDL that induce TLR4-dependent macrophage activation. The TLR4 downstream signaling pathway induced by mmLDL and oxidized CE involves recruitment of spleen tyrosine kinase (Syk) to a TLR4 signaling complex, TLR4 phosphorylation, activation of a Vav1-Ras-Raf-MEK- ERK1/2 signaling cascade, phosphorylation of paxillin, and activation of Rac, Cdc42 and Rho. These oxidized CE-induced and TLR4-dependent signaling events and cytoskeletal rearrangements lead to enhanced uptake of small molecules, dextran and, most importantly, of both native and oxidized LDL, resulting in intracellular lipid accumulation. Remarkably, the oxidized CE molecular species first identified in mmLDL were also found in lipid extracts from mouse atherosclerotic lesions. These data describe a novel mechanism leading to enhanced lipoprotein uptake in macrophages that would contribute to foam cell formation and atherosclerosis. These data also suggest that polyoxygenated CE hydroperoxides are an endogenous ligand for TLR4. As TLR4 is highly expressed on the surface of circulating monocytes and tissue macrophages in patients with chronic inflammatory conditions, and oxidized CE are present in plasma and in atherosclerotic lesions, the oxidized CE-induced and TLR4-dependent activation of macrophages and lipid uptake may contribute to monocytes and macrophages pathological roles in chronic inflammatory diseases.

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36. Oxidized cholesteryl esters and phospholipids in zebrafish fed a high-cholesterol diet: Macrophage binding and activation

Longhou Fang[1], Richard Harkewicz[2], Karsten Hartvigsen[1], Tiffany Sayaphupha[1], Edward A. Dennis[2], Joseph L. Witztum[1], Sotirios Tsimikas[1], Yury I. Miller[1]

Departments of [1]Medicine, [2]Pharmacology,and [2]Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093.

A novel hypercholesterolemic zebrafish model has been developed to study early events of atherogenesis. This model utilizes optically transparent zebrafish larvae, fed a high- cholesterol diet (HCD), to monitor processes of vascular inflammation in live animals. Because lipoprotein oxidation is an important factor in the development of atherosclerosis, in this study, we characterized the oxidized lipid milieu in HCD-fed zebrafish larvae. Using liquid chromatography-mass spectrometry, we show that feeding a HCD for only 2 weeks resulted in up to 70-fold increases in specific oxidized cholesteryl esters, identical to those present in human oxidized LDL (OxLDL) and in murine atherosclerotic lesions. The levels of oxidized phospholipids (OxPL), such as 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3-phosphocholine and various lysophosphatidylcholines, were also significantly elevated. Using biotinylated homogenates of HCD-fed larvae, we demonstrated that their components bound to murine macrophages, and this binding was effectively competed by minimally oxidized LDL, but not by native LDL. Homogenates of HCD-fed larvae induced membrane ruffling and cell spreading as well as ERK1/2, Akt and JNK phosphorylation in murine macrophages. These effects were effectively inhibited by immunodepletion of the homogenates with antibodies against OxPL or apoB, as well as by reducing lipid hydroperoxides with ebselen. These data suggest that HCD feeding leads to extensive accumulation in zebrafish of biologically active oxidized lipid moieties, identical to those found in mammalian OxLDL and atherosclerotic lesions. Collectively, these results support future studies of hyper- cholesterolemic zebrafish as a novel model for studying the role of oxidized lipoproteins in the early events of atherogenesis.

37. Mapping the *M. tuberculosis* lipidome reveals previously unknown deoxymycobactins*

Sunhee Hong[1], Cressida A. Madigan[1], Tan-Yun Cheng[1], David Young[1], Emilie Layre[1], Lindsay Sweet[1], John Annand[1], Rahul S. Talekar[2], Matthew McConnell[1], Anthony C. Debono[3], Isamu Matsunaga[4], D. Branch Moody[1]

[1]Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA. [2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA. [3]Department of Biology, Technician at the Massachusetts Institute of Technology, Cambridge, MA 02139, USA. [4]Department of Viral Oncology, Institute for Virus Research, Kyoto University. Sakyo-ku, Kyoto 606-8507, Japan.

Over 1.8 billion humans are currently infected with *M. tuberculosis*, an organism that causes more than 1.5 million deaths annually. *M. tb*'s invest 6 percent of its genome in lipid modifying enzymes, creating a unique cell wall structure with 2 lipid bilayers that controls drug and metabolic uptake during its decades long infection cycle. We developed a new high-throughput liquid chromatography (LC)- quadrupole time-of-flight (QToF) high-mass accuracy spectrometry (MS) platform that is capable of detecting and analyzing up to 10,000 unique lipid species in a single experiment. Currently, there is no comprehensive database available denoting the specific classes and species of both secretory and intrinsic cell wall lipids produced by *M. tuberculosis*. In addition, we have developed a software program that is able to group compounds as homologous series based on mass intervals, such as those corresponding to methylene groups, sugar units, or phosphate groups. *M.tb* growth in hosts requires iron, which it obtains using the lipidic virulence factors mycobactin and its secreted form, carboxymycobactin. After the unexpected discovery of dideoxymycobactin, an antigenic dehydroxylated mycobactin variant (*Science* 303, p. 527), we interrogated our libraries of cell envelope and secreted lipids to test a new model of siderophore biosynthesis. We rapidly discovered 42 previously unknown deoxysiderophores, designated monodeoxymycobactins and monodeoxycarboxymycobactins. Together with genetic analysis of dideoxymycobactin production during iron deprivation, these deoxysiderophores support a new model of the biosynthesis of mycobactin and carboxymycobactin. The new model proposes lower iron-affinity deoxysiderophores are first produced and then finally activated by hydroxylation to a high iron-affinity form for iron transport.

38. Determination of oxidized neutral lipids in human atherosclerosis

Patrick M. Hutchins, Robert M. Barkley, Robert C. Murphy

Department of Pharmacology, University of Colorado Denver.

Atherosclerosis, a leading cause of death in the western world, results in remodeling of the arterial wall, which is characterized by massive accumulation of both intracellular and extracellular lipids. The oxidative modification hypothesis postulates that oxidation of low-density lipoprotein (LDL) triggers an inappropriate innate immune response which is the fundamental process underlying atherogenesis. Oxidized LDL (oxLDL), its bioactivity, and the protein and phospholipid components of oxLDL have been the subject of many studies, while relatively little attention has been paid to related oxidized neutral lipids.

Neutral lipids were extracted from atheroma, plasma or lipoprotein fractions and separated by normal phase chromatography using gradient elution. Electrospray ionization mass spectrometry was used to detect cholesteryl esters (CE) by precursor ion scanning, while glycerolipids, including triacylglycerols (TAG), were detected using full mass scanning.

Oxidized cholesteryl ester (oxCE) species were detected in both human plasma and atheroma samples. Interestingly, endogenous oxidized triacylglycerides were not observed in either plasma or atheroma. Initial characterization of oxCE species suggested that there were numerous CE oxidation products present in both blood and atheroma, including some species that had not been previously reported. The analysis of atheroma samples revealed abundant oxCE species containing 1, 2 or 3 oxygen atoms covalently linked to the polyunsaturated fatty acyl moiety. The exact structure of these species is under investigation using MS/MS techniques. The most abundant of these oxCE appeared to result from oxidation of the most abundant CE species: cholesteryl linoleate and cholesteryl arachidonate. Curiously, oxidation products of cholesteryl docosahexanoate were detected only at very low levels despite this CE being relatively abundant in human plasma and atheroma. The relative abundance of these oxCE species contained in atheroma samples, compared to plasma or normal tissue, suggests that the degree of cholesteryl ester oxidation increases dramatically during atherogenesis. Finally, initial results also indicated that LDL and/or VLDL particles may be the chief transport vesicles for oxCE in human plasma.

39. PAR signaling in platelets activates cPLA$_2$-α differently for COX-1 and 12-LOX catalysis

Olivier Boutaud[1], Michael Holinstat[2], Heidi E. Hamm[1], John A. Oates[1,3]

Departments of [1]Pharmacology and [3]Medicine, School of Medicine, Vanderbilt University, Nashville, TN, USA. [2]Departments of Medicine, and Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA, USA.

Thrombin-regulated platelet activation mediates a range of vascular effects, which includes thrombus formation. Platelet activation with thrombin leads to formation of eicosanoid mediators derived from two metabolic pathways: cyclooxygenase-1 and 12-lipoxygenase. These two enzymes share a common substrate, arachidonic acid, and we have previously determined that cPLA$_2$-α is the enzyme responsible for more than 95 % of production of both of these eicosanoids in human platelets. We investigated the production of thromboxane A$_2$ (TxA$_2$) and 12-hydroxyeicosatetraenoic acid (12-HETE) in response to selective activation of the two thrombin receptors, PAR1 and PAR4. Our data suggest that production of TxA$_2$ precedes production of 12-HETE in response to PAR agonists and that PAR-induced 12-HETE production is TxA$_2$-dependent. We observed that inhibition of COX-1 by aspirin leads to a decrease in 12-HETE formation following activation of PAR1 but to the opposite effect when PAR4 is activated. Investigation of the lipid pathways regulating cPLA$_2$-α indicated that an inhibitor of phosphatidylinositol kinases that blocks formation of both PI(4,5)P2 and PI(3,4,5)P3 prevents activation of the cPLA$_2$-α coupled to TxA$_2$ production but this pathway is not involved in activation of the cPLA$_2$-α coupled to 12-HETE synthesis. Products from the PLD pathway also differentially regulated production of the two eicosanoids. These results suggest that both PAR1 and PAR4 contribute to TxA$_2$ and 12-HETE formation caused by thrombin activation but that each PAR utilizes a different signaling pathway in order to achieve full eicosanoid formation. It also indicates that phosphatidyl inositol kinases and phospholipase D are important for TxA$_2$ but not for 12-HETE production. Taken together, this evidence suggests that the mechanism for activating the cPLA$_2$-α-mediated arachidonic acid release that generates TxA$_2$ is quite different from that which provides substrate for the 12-lipoxygenase. *This study was supported by grants P50 HL081009 and R00 HL089457 from the NIH.*

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40. Discovery of novel non-cytidine liponucleotides in *S. cerevisiae* and *E. coli*

Gregory M. Laird, Ziqiang Guan, Reza Kordestani, Christian R.H. Raetz

Department of Biochemistry, Duke University Medical Center, P.O. Box 3711, Durham, North Carolina 27710, USA.

CDP-diacylglycerol (CDP-DAG) is a key intermediate in phospholipid biosynthesis, acting as the source of the phosphatidyl group for all major phospholipids of *E. coli* and the anionic phospholipids of most eukaryotes. In *E. coli*, CDP-DAG is formed by the inner membrane protein CDP-DAG synthetase (cdsA), which serves to activate phosphatidic acid (PA) via the addition of cytidine monophosphate from donor CTP. Enzymatic studies of cdsA have been carried out in *E. coli* and illustrate high substrate specificity for CTP *in vitro*, with other nucleotide triphosphates yielding no detectable reaction product. Using highly sensitive HPLC mass spectrometry, we have detected a number of novel liponucleotides in *S. cerevisiae* and *E. coli*. In an *S. cerevisiae* cardiolipin synthase (crd1) knockout, we have detected massive accumulations of the novel liponucleotides ADP-DAG and UDP-DAG. These and other liponucleotides were also detected in *E. coli* lipid extracts. CDP-DAG is known to be utilized in *E.* coli by the phosphatidyl transferases phosphatidylserine synthase (pss) and phosphatidylglycerophosphate synthase (pgsA). Experiments in radiolabeled cells suggest that both pss and pgsA are highly specific for CDP-DAG as a substrate. To determine the role of non-cytidine liponucleotides in the cell, we first sought to determine their rate of formation and turnover using a pulse-chase experiment. The *E. coli* strain BB20-14 was used, given that it is dependent upon glycerol or glycerol-3-phosphate supplementation for phospholipid biosynthesis and thus can be utilized in liponucleotide labeling experiments with labeled glycerol. We have determined that non-cytidine liponucleotides label at rates comparable to that of CDP-DAG, suggesting comparable rates of formation for cytidine and non-cytidine liponucleotides. We have also determined that non-cytidine liponucleotides turnover over rapidly. Further studies into the function of non-cytidine liponucleotides are ongoing.

41. Exploring lipid-protein interactions: identification of endogenous ligands of CD1

Ziqiang Guan[1], Neil Haig[2], Xiao-ning Xu[2], Andrew J. McMichael[2], Christian R. H. Raetz[1]

[1]Department of Biochemistry, Duke University Medical Center, Durham, NC 27710. [2]Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, UK.

CD1 (cluster of differentiation 1) is a family of glycoproteins expressed on the surface of various antigen-presenting cells. In contrast to the classical major histocompatibility complex (MHC) molecules which present peptide antigens, CD1 molecules present cellular and pathogen-derived lipid antigens to specific T lymphocytes.

Although the structural roles of CD1 molecules in presenting lipid antigens to the T cell receptors have been well established, much remains unknown about the CD1 synthesis/assembly in the ER, trafficking to the endosomes and subsequent antigen loading. For example, how is the large hydrophobic groove in CD1 maintained before antigen binding? Are there any endogenous lipids bound to CD1 for its proper folding? Furthermore, what are the endogenous lipids that may serve as CD1-presented antigens, and are they involved in the positive selection of CD1-restricted T cells? To address these questions, it is important to identify the endogenous lipids that directly bind/interact with CD1 proteins in cells. Experimentally, we focused on CD1d, one of the four CD1 isoforms, and sought to identify the lipids that specifically bind to and may co-purify with CD1d.

To overcome the difficulties of extracting CD1d (a membrane protein) and the limitations of traditional methods for lipid analysis, we developed an approach that includes expression and purification of a soluble form of CD1d (devoid of detergents) in large quantities (milligrams), followed by lipid extraction and highly sensitive HPLC/MS/MS analysis. The CD1d-bound lipids include sphingomylins, sufatides and several gangliosides. Some have been used, via CD1d tetramer staining, to identify novel populations of antigen-specific T cells from fresh peripheral blood mononuclear cells (PBMCs). This strategy may be adapted to identify pathogen-derived lipids that are presented during infections.

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42. Measurement of bioactive lipids using immunoassays and receptor interaction methodologies

Kelli Moreno, Jon Wojciak, Rosalia Matteo, Mary-Ann Campbell, Roger Sabbadini

Lpath Inc., San Diego, CA 92121, USA.

Biologically active lipids are important mediators of cellular signaling and regulation, whereby abnormal levels of these molecules may lead to pathological processes such as cancer, inflammatory and neurodegenerative diseases and dysfunctional fibrosis. Identifying aberrant levels of bioactive lipids in disease states is essential for elucidating the pathologies and developing treatment strategies. Lpath, Inc. is a biotechnology company focused on discovery and development of lipid-based therapeutics and diagnostics. Lpath's proprietary ImmuneY2™ technology allows the company to generate monoclonal antibodies against extracellular lipid signaling mediators and neutralizes them by molecular absorption. Lpath has developed a first-in-class therapeutic agent, a humanized monoclonal antibody Sonepcizumab™ (or LT1009), which was derived from the murine form of the antibody, Sphingomab™. Sonepcizumab neutralizes the bioactive lipid signaling mediator, sphingosine-1-phosphate (S1P). S1P contributes to disease in cancer, multiple sclerosis, inflammatory disease and ocular diseases that involve dysregulated angiogenesis. Here we describe several methodologies that make use of anti-S1P antibodies to characterize S1P in biological fluids. We developed a quantitative enzyme-linked immunosorbent assay (ELISA) along with other antibody-based assays to quantify S1P in both healthy and diseased samples. The S1P concentrations measured using these assays are similar to levels measured using traditional techniques such as LC-ESI MS/MS. The ELISA format can be used for high throughput analysis without lipid extraction. Finally, we present preliminary data characterizing the interaction between plasma S1P and its cognate G protein-coupled receptor, S1P1, using the β-Arrestin PathHunter™ technology.

43. Characterization of an ASAH1-knockdown H295R stable cell line: A role for nuclear ASAH1 in steroidogenic gene transcription

Natasha C. Lucki[1], Marion B. Sewer[2]

[1] School of Biology, Georgia Institute of Technology, Atlanta, GA. [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA.

Sphingolipids have been implicated in various cellular processes. Acid ceramidase (ASAH1) catalyses the degradation of ceramide (cer) into sphingosine (SPH) and a free fatty acid. In H295R adrenocortical cells, we have previously demonstrated that adrenocorticotropin (ACTH), the primary regulator of cortisol biosynthesis in the human adrenal cortex, induces the transcription of ASAH1 and rapid metabolism of several sphingolipid species, including cer. In addition, we have identified SPH as an antagonist ligand for the nuclear receptor steroidogenic factor-1 (SF-1), a transcriptional regulator of most steroidogenic genes. Based on the predominantly nuclear localization of SF-1, we hypothesized that ACTH signaling activates local sphingolipid metabolism in the nuclei of adrenocortical cells. To test this hypothesis we assessed the nuclear expression of various sphingolipid enzymes including ceramidases (ASAH1-3), ceramide synthases (LASS1-6), neutral sphingomyelinase (nSMase), and sphingosine kinase 1 and 2 (SK1/2) and found that some enzymes are expressed in the nucleus. In addition, ASAH1 directly binds to SF-1 and regulates the transactivation potential of the receptor. To further define the role of nuclear sphingolipid metabolism in regulating SF-1 function, we developed an H295R cell line that stably expresses a short-hairpin RNA (shRNA) against ASAH1 under the regulation of tetracycline (Tet). Tet-treated ASAH1 shRNA stable cells express significantly reduced ASAH1 mRNA and protein levels by 64% and 75% of untreated controls, respectively. ASAH1 knockdown increased the expression of multiple steroidogenic genes, including CYP17, CYP11A1, and steroidogenic acute regulatory protein (StAR). In addition, mass spectrometric analyses of whole cells and nuclei revealed that reduced ASAH1 expression alters the pools of ceramides. Collectively, these studies demonstrate that ACTH signaling regulates steroidogenesis by evoking acute, dynamic changes in nuclear pools of bioactive sphingolipids and that ASAH1 modulates steroidogenic gene transcription by interacting with SF-1.

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44. Searching the LIPID MAPS databases

Dawn Cotter[1], Eoin Fahy[1], Manish Sud[1], Shankar Subramaniam[1,2]

[1]LIPID MAPS Bioinformatics Core, SDSC, [2]Department of Bioengineering, University of California, San Diego, La Jolla, CA, 92093.

Given the importance of these molecules in cellular function and pathology, it is essential to have a well-organized database of lipids and lipid-related genes and proteins that is extensible, flexible and scalable. An object-relational database of lipids, the LIPID MAPS Structure Database (LMSD) is based on an internationally accepted classification system and is the largest public lipid-specific repository in the world with over 22,000 individual structures. The LIPID MAPS Proteome Database (LMPD) was developed to provide a catalog of genes and proteins involved in lipid metabolism and signaling. We present extensive browsing and searching tools available on the LIPID MAPS website for these and other lipid-specific resources.

45. Tools for lipidomics: mass spectrometry prediction and structure drawing methods

Dawn Cotter[1], Eoin Fahy[1], Manish Sud[1], Shankar Subramaniam[1,2]

[1]LIPID MAPS Bioinformatics Core, SDSC, [2]Department of Bioengineering, University of California, San Diego, La Jolla, CA, 92093.

Certain classes of lipids such as glycerolipids, glycerophospholipids and sphingolipids which are composed of an invariant core and one or more acyl/alkyl substituents are good candidates for MS computational analysis since their molecular ion masses can usually be predicted on the basis of permutations of commonly occurring side-chains and also because they tend to fragment predictably in EIMS leading to detection of diagnostic product ions. It is possible to create a virtual database of permutations of the more common side-chains for various lipid categories and calculate “high-probability” product ion candidates in order to compare experimental data with predicted spectra. The LIPID MAPS group has developed a suite of search tools allowing a user to enter an m/z value of interest and view a list of matching structure candidates, along with a list of calculated “high-probability” product ions. These search interfaces have been integrated with structure-drawing and isotopic-distribution tools. A suite of structure-drawing tools has been developed and deployed which dramatically increase the efficiency of generating large and complex lipid structures, facilitate data entry into lipid-structure databases and permit “on-demand” structure generation in conjunction with a variety of mass spectrometry–based informatics tools.

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46. LIPID MAPS methodologies for pathway construction and analysis

Robert Byrnes[1], Dawn Cotter[1], Eoin Fahy[1], Shakti Gupta[1], Sean Li[1], Mano Maurya[1], Manish Sud[1], Shankar Subramaniam[1,2]

[1]LIPID MAPS Bioinformatics Core, SDSC, [2]Department of Bioengineering, University of California, San Diego, La Jolla, CA, 92093.

The LIPID MAPS bioinformatics group has developed several tools for managing, visualizing, and editing signaling and metabolic pathways related to lipid biology. These have been applied to the study of the extensive lipidomics and transcriptomic data generated from integrated studies on macrophages subjected to a variety of treatments. We present software and tools for construction and visualization of lipid-related pathways.

47. A strategy for the quantitative analysis of all lipids with a single acquisition method

Sahana Mollah, Brigitte Simons, Stephen Tate

AB SCIEX, Canada.

Introduction: Potential biomarkers could potentially be identified in complex lipid extracts without a need for extensive fractionation or method development. To this end, the comprehensive study of lipids is limited and analytically challenging but perhaps overcome by the use of either accurate mass for lipid identification and use of precursor ion scans for the analysis of different functional groups. The presentation describes a new analytical technique for identifying and quantifying lipids in a single fast acquisition.

Methods: Lipid extractions of rat brain tissue were carried using standard techniques and samples were standardized for total amount of lipid (~20μM), lipid internal standards were added to a final concentration of 0.2 μM, and diluted appropriately into chloroform and methanol [1:2 (v/v)] with 5 mM ammonium acetate. Direct Nano-electrospray infusion was carried out using an automated chip-based nanoESI source where a 10 μL infusion of lipid extract can enable the acquisition of the complete quantitative and qualitative method. MSMS acquired for every mass between 200 and 1000 amu. This data was stored in a 3D datafile which consists of one dimension for time, one for precursor selection mass and one for daughter ion mass.

Preliminary data: Through the nanoelectrospray infusion analysis of very small lipid extract volumes, global lipid profiling experiments shows the identification of lipid species from 6 different lipid classes and 15 sub-classes encompassing a total of over 800 lipid species identified in a single polarity specific acquisition. Precursor Ion Scanning in both positive and negative modes generated complex lipid array data with an extensive list of 43, fully characterized, fatty acid moieties. This single acquisition approach offers reproducible quantitation when measuring the response of lipid species against their lipid class –specific internal standard through a wide dynamic range.

Novel aspect: This study presents a simple quantitative workflow for the identification of hundreds of lipids in total lipid extracts from rat brain tissue.

48. Lipidomic analysis of bioactive lipids in Chronic Lymphocytic Leukaemia

Mojgan Masoodi[1], David Spaner[2], Albert Koulman[1], Michael Eiden[1], Dietrich A. Volmer[1]

[1]Lipidomic and biomarker research, Medical Research Council, Elsie Widdowson Laboratory, Cambridge, UK. [2]Division of Molecular and Cellular Biology, Sunnybrook Research Institute, Toronto, ON, Canada.

Eicosanoids are a class of bioactive lipids derived from polyunsaturated fatty acids (PUFA) via COX, LOX, cytochrom P450 and other non-enzymatic pathways. They exhibit a wide range of tissue and cell-specific activities in many physiological and pathological processes. Since these metabolites are produced within the same cascade and are part of a complex regulatory network, it is necessary to profile all metabolites simultaneously. However, identification and quantification of this class of compounds are challenging tasks because many of these compounds are isomers with similar chemistry and physical properties. Additionally, biological samples contain very low levels of eicosanoids and therefore a comprehensive study of this class of lipids requires a highly sensitive and selective analytical method. Simultaneous characterisation of up to 80 lipid mediators, including derivatives of linoleic, arachidonic, eicosapentaenoic and docosahexaenoic acids, was successfully performed by hyphenated liquid chromatography orbitrap mass spectrometry with high mass accuracy and resolving power. High resolution chromatography was performed using an acetonitrile-based linear gradient reversed-phase at 0.7 ml/min flow rate over 10 min. The dynamic range of the assay was linear over the range of 10-200 pg/ml. Automated data-dependent functions were used to acquire precursor ions from a generated ion list. To demonstrate the applicability of this approach this system was applied to profile these bioactive lipids in Chronic Lymphocytic Leukaemia (CLL) cells. Our application readily demonstrates that the use of high mass accuracy MS^2 scans can be used effectively to detect unknown bioactive lipids in biological samples without necessitating standard compounds and our preliminary data suggest that the alteration in monohydroxy fatty acids levels may have pathogenic significance in CLL. This method is useful for profiling a range of biochemically-related potent mediators and also facilitates structural illumination and discovery of novel lipid mediators. Thus, this system provides a comprehensive lipidomic approach for identification of potential lipid mediators in relation to disease pathology and may facilitate future therapeutic approaches.

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49. Profiling and imaging of phospholipids in rat brain sections after ischemic stroke*

Hay-Yan J. Wang, Cheng Bin Liu, Hsuan-Wen Wu

Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan.

Ischemic stroke, a potentially deleterious cerebrovascular event, is characterized by a critical reduction of blood flow to the brain parenchyma. The ensuing inflammatory responses and apoptosis result in severe loss of brain tissue and brain function. The release of pro-inflammatory lipid mediators after stroke is closely associated with the breakdown of phospholipids. We characterized such phospholipid changes in rat brains 24 hours after ischemic stroke by direct profiling and imaging using matrix-assisted laser desorption-ionization-mass spectrometry (MALDI-MS). The elevated level of tissue sodium in the infarcted area was manifested by a shift of phospholipid profile from a mostly protonated and potassiated pattern to a largely sodiated pattern. MALDI imaging verified such a shift in phospholipid pattern. The delineated infarcted area by this feature appeared similar to those seen in the regular histology preparation. An in situ desalting method was also applied on the brain sections to remove the interference of sodium and potassium before profiling and imaging. After desalting, the mass profile clearly showed a roughly 50% reduction of brain PC 34:1 and PC 38:6 in the infarcted region that was previously masked by their corresponding sodiated species. Highly elevated lyso-PC 16:0 (LPC 16:0), LPC 18:1, LPC 20:4, and LPC 22:6 in tissue were also noted in the infarcted area, albeit along the core region of infarction. Most of the MALDI imaging results corroborate and complement the profiling observations. However, MALDI-imaging also showed the paradoxical increase of some stroke-affected minor PC species in the infarcted area that may be attributed to the change of parenchymal density by stroke-induced edema. In summary, the knowledge of brain phospholipid dynamics after stroke nonetheless suggests some intervening approaches that may carry therapeutic potential for the management of stroke and acute brain injuries.

50. In situ desalting that assists the direct MALDI-MS profiling of tissue phospholipids

Hay-Yan J. Wang, Hsuan-Wen Wu, Cheng Bin Liu, Hsuan-Po Liu

Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan.

In situ profiling of phospholipids by matrix-assisted laser desorption-ionization mass spectrometry (MALDI-MS) usually generates a snapshot of the phospholipid profile in protonated, sodiated, and potassiated forms. However, the cationization of phospholipids often interfere with direct visualization of the relative abundance of in situ phospholipid species. Some sodiated phospholipids also pose as the isobaric interference to certain protonated phospholipids. In this study we tested a simple in situ desalting approach to eliminate the interference of cations to the phospholipids. Rat brain sections were gently washed with 70% ethanol (EtOH), doubly-distilled water (ddH2O), or ammonium acetate solution (NH4Ac) and immediately vacuum-dried. The tissue phospholipids were direct profiled by MALDI-MS. The total lipids in the washing runoffs were extracted, reconstituted, and examined by MALDI-MS. Desalting by EtOH or ddH2O only partially removed the potassium and sodium adducted to the phospholipids in tissue. A significant amount of phospholipids was detected in the washing runoffs of both preparations. Brain sections desalted with NH4Ac demonstrated a tissue phospholipid profile that was devoid of potassiated and sodiated species, and no phospholipid was detected in the washing runoff of this preparation. The NH4Ac wash was further tested on the sections cut from the rat brain slices stained with 0.1% triphenyltetrazolium chloride (TTC). The NH4Ac wash successfully removed the interfering signal at m/z 633 which heavily suppressed the tissue phospholipid signals, yet without altering the staining characteristics of TTC to the brain sections. The results demonstrate that NH4Ac desalting is a viable processing method that simplifies the tissue phospholipid profile, enhances the ion signals, and facilitate their identification. This approach may couple with direct profiling or MALDI imaging to reveal the underlying tissue phospholipid changes in pathological conditions where the interference by the surge of interstitial sodium due to pathological change is prominent.

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51. Quantitative proteomic analysis of the effect of 24(S),25-epoxycholesterol on neuronal cells

Ian R. Gilmore[1], Jenny Ho[2], Martin Hornshaw[2], William J. Griffiths[1], Yuqin Wang[1]

[1]Institute of Mass Spectrometry, School of Medicine, Swansea University, Swansea, UK. [2]Thermo Fisher Scientific, Hemel Hempstead, UK.

Oxysterols have recently been implicated in increased neurogenesis in the ventral mid brain region of embryonic brain; an area with a high density of dopaminergic neurons. Loss of neurons in this area can lead to Parkinson's disease; a disease in which there is some evidence that disturbed cholesterol homeostasis plays a role. Recently higher than expected levels of the LXR ligand 24(S),25 epoxycholesterol (24S,25-EC) have been identified in murine embryonic mid brain. Therefore, here we investigated the effect of 24S,25-EC on protein expression in a mouse substantia nigra dopaminergic cell line (SN4741) using a stable isotope labelling (SILAC) quantitative proteomic approach. SN4741 cells were cultured in SILAC media containing differentially isotope labelled arginine and lysine. Cells were treated in serum free SILAC media with vehicle, 10μM 24S,25-EC, or 1μM GW3965 (a synthetic LXR agonist) for 24 hours. Cells were lysed before being combined in a 1:1:1 (light:medium:heavy) ratio. Protein was trypsin digested before peptide separation via strong cation exchange chromatography. Resulting fractions were analysed using LC-MS/MS on a LTQ-Orbitrap Velos over a 120 min gradient using a 'top 20' data dependent method. Data was quantified using MaxQuant software in conjunction with Mascot using an IPI mouse database. Over three biological replicates a total of 6785 proteins were identified with >2 peptides and quantified, of these 1575 were identified in all three experiments. Known LXR and SREBP2 regulated genes were differentially expressed in all three experiments; the LXR regulated cholesterol transport protein ABCA1 was up-regulated whilst members of the cholesterol biosynthesis pathway, and the LDL receptor were down-regulated. Unexpectedly, ethanolamine-phosphate cytidyltransferase was down-regulated in an LXR independent manner. We also observed a number of membrane and transport proteins affected by treatment with 24(S),25-EC. This result implicates that 24(S),25-EC has a function beyond cholesterol homeostasis with effects on membrane composition and signalling.

52. Analysis of oxysterols and cholestenoic acids in plasma and CSF by charge-tagging and LC-MS^{n}⋆

William J. Griffiths[1], Michael Ogundare[1], Andrew Lockhart[2], Jan Sjövall[3], Yuqin Wang[1]

[1]Institute of Mass Spectrometry, School of Medicine, Swansea University, Singleton Park, Swansea, SA2 8PP, UK. [2]Translational Medicine, GlaxoSmithKline R&D China, Addenbrookes Hospital, Cambridge, CB2 2GG, UK. [3]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, SE-17177, Sweden.

Oxysterols and their down-stream acidic C_{27} metabolites were once thought of as transport forms of cholesterol whose main function was return of sterol to the liver for metabolism to C_{24} bile acids. In recent years it has been realised that some oxysterols and C_{27} acids are bioactive molecules in their own right, acting as ligands to nuclear receptors e.g. LXR, FXR, PXR, and also INSIG. However, structure activity relationships appear to be different in different cell types and it is unclear whether all ligands identified in vitro are also ligands in vivo.

Here we present a method for the high-sensitivity analysis of oxysterols and C_{27} acids. The method is based on charge-tagging of a quaternary nitrogen containing group to the steroid ring. The method is selective for steroids with 3β-hydroxy-5-ene, 3β-hydroxy-5α-hydrogen or oxo group and offers improvement in sensitivity upon analysis by ESI-MS of two – three orders of magnitude. By performing MS^3 structural information is forthcoming. Using this methodology we have identified the following free oxysterols and C_{27} acids in adult and infant plasma; 24S-hydroxycholesterol (7 – 30 ng/mL); 25-hydroxycholesterol (<6 ng/mL); 27-hydroxycholesterol (21 – 26 ng/mL); 7α-hydroxycholesterol (2 – 8 ng/mL); 7α,27-dihydroxycholesterol/7α,27-dihydroxycholest-4-en-3-one (6 – 11 ng/mL); 7α,25-dihydroxycholesterol/7α,25-dihydroxycholest-4-en-3-one (< 3 ng/mL); 3β-hydroxycholest-5-enoic acid (90 – 152 ng/mL); 3β,7α-dihydroxycholest-5-enoic/7α-hydroxy-3-oxocholest-4-enoic acid (60 – 164 ng/mL). Levels of free oxysterols and C_{27} are much lower in CSF (e.g. 24S-hydroxycholesterol 0.018 ng/mL; 3β-hydroxycholest-5-enoic acid 0.416 ng/mL). However a dihydroxy-3-oxocholest-4-enoic acid (1.330 ng/mL) and a 7α-hydroxy*bis*oxocholest-4-enoic acid (0.063 ng/mL), probably 7α,24-dihydroxy-3-oxocholest-4-enoic and 7α-hydroxy-3,24-*bis*oxocholest-4-enoic acids, respectively were found exclusively in CSF. This data suggest that these two acids are formed in brain and represent end products of 24S-hydroxycholesterol metabolism in brain. Others have suggested that levels of 24S-hydroxycholesterol in CSF may represent a marker of cholesterol homeostasis in brain, and a biomarker for neurodegenerative disease. Our results suggest that 7α,24-dihydroxy-3-oxocholest-4-enoic and 7α-hydroxy-3,24-*bis*oxocholest-4-enoic acids should represent better markers.

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53. SIMS and MALDI imaging of glycerophospholipid

Melissa K. Passarelli, Nicholas Winograd

Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, PA 16802.

SIMS and MALDI methodologies were used to investigate the variety and distribution of lipid species in biological materials, such as tissues and cells. Characterizing the lipid content of biological material with mass spectrometry directly without utilizing extraction, purification and separation techniques is often impeded by isobaric interference. Overlapping peaks in the lipid region produce convoluted spectra and make it difficult to directly identify and quantify individual lipid species. To help mitigate these issues, we utilize the simplicity of MALDI spectra, common motifs in lipid profiles, and tandem MS to deconvolute lipid profiles. High resolution SIMS images were obtain from native tissue and compared to tissue interrogated with a matrix. For SIMS-based analysis matrices were sublimated[1]— 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid — and evaporated—glycerol and trifluoroacetic acid (TFA)—on the tissue sample to enhance secondary ion yields. The same matrix application technique was employed for MALDI based analyses. With these techniques, we examined brain tissue (Sprague-Dawley rat) with a fluid percussion-based brain injury, an experimental model for traumatic brain injury. Imaging mass spectrometry revealed increased potassium-lipid adducts at the injury site. We also examined ganglia tissue and cells obtained from *aplysia californica,* a sample with a simple well-define neural network. A major lipid component was identified at m/z 746 to be 1-O-hexadecyl-2-oleoyl-*sn*-glycero-3-phosophocholine (16:0e/18:1 GPCho). With MALDI and SIMS, we were able to identifying major lipid and visualize its distribution across the surface of a single neuron.

[1] J. A. Hankin, R. M. Barkley, R. C. Murphy, Journal of the American Society for Mass Spectrometry 2007, 18, 1646.

54. Effects of inflammatory cytokines on sulfoglucuronosyl paragloboside expression in human cerebromicrovascular endothelial cells

Robert K.Yu, Somsankar Dasgupta, Guanghu Wang

Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA 30912.

The expression of sulfoglucuronosyl paragloboside (SGPG), a sulfated glycosphingolipid, was elevated in human cerebromicrovascular endothelial (SV-HCEC) cells after exposure of these cells to inflammatory cytokines such as TNFα and IL-1β. Such elevation was mediated by stimulation of the expression of glucuronosyltransferase (P and S) genes that are responsible for SGPG synthesis, and the increased SGPG expression in the cells promoted T cell adhesion [Dasgupta *et al*. J. Neurosci. Res. 85:1086, 2007]. Inhibition of SGPG synthesis by silencing the gene HNK-1 sulfotransferase (HNK-1ST) down-regulated SGPG expression, resulting in reduced T ($CD4^+$) cell adhesion. HNK-1ST siRNA-transfected cells displayed down-regulation of NFκB signaling even after TNFα/IL-1β stimulation [Dasgupta *et al*. J. Neurosci. Res. 2009, 87:3591]. The transfected cells also became less permeable after TNFα exposure compared with controls. Additionally, HNK-1STsiRNA transfection offered partial protection to SV-HCECs from TNFα/IL-1β-mediated apoptosis by elevating ERK activation and reducing the caspase 3 activity. Apoptosis was further determined using TUNEL assay. Understanding the precise regulation of SGPG expression via NFκB signaling should enable us to design novel therapeutic agents for neural inflammation. (Supported by NIH grants RO1 NS11853-34 and RO1 NS26994-20 to RKY.)

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55. Inhibition of sodium ion channel activity by anti-lipid A antibody of chicken with campylobacteriosis

Robert K. Yu[1], Seigo Usuki[a], Yoshihiko Nakatani[2], Kyoji Taguchi[2], Toshio Ariga[1]

[1]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA; [2]Department of Neuroscience, Showa Pharmaceutical University, Tokyo, Japan.

A microbiologically confirmed strain VLA2/18 of *Campylobacter jejuni* (non-serotyped) was isolated from a patient with campylobacteriosis. The serum of chicken infected by VLA2/18 contained a high-titer anti-Lipid A antibody (anti-Lipid A Ab) against Lipid A from VLA2/18. The innate immune systems of humans and other animals are activated by lipopolysaccharides (LPSs) of Gram-negative bacteria. Kdo2-Lipid A is a minimal structure of Toll-Like Receptor 4 ligand required for activation of human macrophages. The chicken serum also reacted with Kdo2-Lipid A as a cross-reacting antigen. We studied the effect of the chicken anti-Lipid A Ab on voltage-gated Na currents in whole-cell patch-clamped NSC-34 cells. A decrease in Na current was observed, which was dependent only on the anti-Kdo2-Lipid A Ab activity in the chicken serum. Based on experiments using tunicamycin-treated cells, anti-Kdo2-Lipid A Ab was shown to induce a functional inhibition of Na channels (Nav) at the protein portion instead of the N-glycan portion. We hypothesized that certain epitopes of the extracellular domains of Nav channels might cross-react with the anti-Lipid A AB. Using a molecular overlapping method, we showed that certain dipeptides of the extracellular Nav channel domains are homologous to the Lipid A structure. Since the lipooligosaccharides (LOSs) of *C. jejuni* are thought to play an important role in the pathogenesis of Guillain-Barré syndrome (GBS), our finding suggests that the anti-Lipid A Ab, generated in the infected chicken, may cross-react with the dipeptides, resulting in depression of the Nav function to account for some of the neurophysiological changes in GBS. *(Supported by USPHS RO1 NS26994-20 to RKY.)*