estrogen receptor. Overall, these results highlight molecular targets that could play a role in differential trends in lung disease incidence and severity between men and women and their influence by contaminants with hormonal activity.

PS 998 S-Nitrosoglutathione Reductase (GSNOR) Activity Is Differentially Regulated within Macrophage Phenotypes

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Macrophages (MΦ) play a crucial role in innate immunity. Upon stimulation, MΦ are divided by two main phenotypes, classic (M1) and alternative (M2) activation, distinguished by their functional patterns. M1 MΦ produce large amount of NO, which reacts with glutathione (GSH) to form S-nitrosoglutathione (GSNO). GSNO can transnitrosate the thiol residues of proteins to form S-nitrosothiols (SNO), with functional consequences. GSNO reductase (GSNOR) reduces the SNO moiety in the presence of NADH to regenerate GSH and produce hydroxylamine. It is a major regulator of intracellular SNO levels. We hypothesized that GSNOR activity is regulated within MΦ activation in order to regulate SNO accumulation within phenotypic differentiation. We have utilized LPS or IL-4 to stimulate a M1 or M2 phenotype respectively in a range of MΦ cultures including Raw264.7, Raw Blue, bone marrow derived MΦ (BMDM) and alveolar MΦ. Incubation of all MΦ with LPS dramatically increased iNOS expression and NO production in all cell systems. IL-4 induced arginase but not iNOS expression. LPS induced M1 MΦ had increased SNO content and SNO-proteins as determined by Cu-Cys reduction assay and biotin-switch. IL-4 reduced total intracellular SNO content and SNO-proteins. LPS treatment decreased MΦ GSNOR expression within 1-6 hours, while IL-4 treatment stimulated expression of GSNOR. These changes were matched by reduced GSNOR activity within cellular lysates. LPS mediated increased in iNOS expression were associated with NF-κB activity in Raw blue cells, and the NF-κB inhibitor CAPE reduced iNOS expression in Raw264.7 cells. However, CAPE had no effect on GSNOR expression, suggesting that GSNOR expression is NF-κB independent. These changes were not specific to cell lines as they were also observed in BMDM and alveolar MΦ. SNO regulation in MΦ in the different phenotypes suggests that SNO plays an important role in MΦ activation. Regulation of GSNOR activity may provide novel therapeutic avenues within inflammatory disease. Supported by NIH HL086621.

PS 999 *Salvia plebeia* Extract Alleviates Inflammatory Response in Murine Arthritis Model and Human Rheumatoid Synovial Fibroblasts

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Salvia plebeia R. Br (*S. plebeia*) has been used for the treatment of a variety of inflammatory diseases and anti-oxidant as an oriental folk medicine. In this study, we investigated the effects of *S. plebeia* extract (SPE) on inflammatory arthritis and underlying mechanisms of action. We used a collagen-induced arthritis (CIA) model in BALB/c mice by immunized with type II collagen. SPE was orally administrated during 5 weeks of arthritis induction. Oral administration of SPE decreased arthritis symptoms such as, physiologic arthritis score, footpad thickness, histopathological changes, in addition to serum IgG1 and IgG2a levels. SPE inhibited Th1/Th2/Th17 phenotype CD4+ T lymphocytes expansion in draining lymph node, expression of inflammatory mediator cytokines, matrix metalloproteinase (MMP)-1 and MMP-3 in the ankle joint tissue. To define the underlying mechanisms of action, tumor necrosis factor-α stimulated rheumatoid arthritis synovial fibroblasts were used. SPE significantly suppressed the expression of inflammatory cytokines and MMP-1 by the down-regulation of nuclear factor-κB and mitogen-activated protein kinases. Taken together, the results indicate that SPE has therapeutic efficacy for chronic inflammatory arthritis, suggesting that SPE might be a candidate for the treatment of RA.

PS 1000 Optimization and Evaluation of Metabolite Extraction Methods for Untargeted Metabolomic Study by LC-QTOF/MS for the Evaluation of Effects of 12-Diindolymethane on RAW 264.7 Murine Macrophages

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Advances in accurate mass spectrometry, better data analysis software and availability of biological pathway databases have allowed for more accurate untargeted metabolomic investigations. The purpose of this study was to optimize the harvesting, extraction and LC-QTOF/MS parameters for the metabolic evaluation of the effects of 12-diindolylmethane (12-DIM) on RAW264.7 murine macrophages. 12-DIM is part of a family of potential anti-inflammatory compounds isolated from Brassica vegetables, such as broccoli, kale and brussel sprouts, and a metabolomics study will evaluate the potential for anti-inflammatory properties of 12-DIM. The optimal harvesting and extraction procedure was quenching with cold methanol and cell scraping, compared to the standard trypsin/ethylenediaminetetraacetic acid (EDTA) treatment. Four different extraction methods were compared to elucidate the highest efficiency for intracellular and extracellular metabolite extraction, including: methanol/chloroform/water, methanol/chloroform/0.1% formic acid, methanol/chloroform and methanol/water. Extraction with cold methanol/chloroform was found to extract the most definable molecular features. The ideal mobile phases were found to be water/0.1% formic acid and methanol, which gave a better chromatographic baseline as compared to acetonitrile. The source parameters including: nebulizer, sheath gas temperature, gas flow, gas temperature and the fragmentor for the LC-QTOF/MS were also optimized. These refined parameters provide a sensitive and reproducible sample preparation and extraction of cellular metabolites from RAW264.7 macrophage cells for a global metabolomics study of the effects of 12-DIM.

PS 1001 Nebulized Thiocyanate Attenuates Inflammation and Oxidative Stress in the Airway and Liver of ENaC Mice

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The pseudohalide thiocyanate (SCN-) has been implicated in innate immune function in mammals but less is known about its impact on inflammation and oxidative stress. SCN- is dysregulated in cystic fibrosis (CF), a disease marked by chronic airway inflammation. Previously, we have shown that nebulized SCN- decreases inflammatory and bacterial burden in B6 mice infected with P. aeruginosa while sparing the antioxidant glutathione (GSH). To investigate the role of SCN- in pathogen-independent inflammation, we administered nebulized SCN- or normal saline vehicle to ENaC mice, a phenotypic model of CF, and wild type littermate controls. Nebulized SCN- significantly decreased airway neutrophil infiltrate concurrent with rescue of the ratio of GSH to oxidized glutathione (GSSG) in airway leukocytes and epithelial lining fluid (ELF) of ENaC mice compared to control. Similarly, nebulized SCN- rescued the GSH:GSSG ratio in lung and liver tissue of ENaC mice to levels comparable to littermate controls. These results demonstrate that SCN- directly impacts pathogen-free airway inflammation and attenuates oxidative stress with both local and systemic protective effects. These results are supported mechanistically by the ability of SCN- to scavenge hypohalous acids, strong oxidants produced by neutrophils, forming a less reactive oxidant (hypothiocyanous acid) that we have previously demonstrated is safely metabolized by mammalian cells. Our findings support nebulized SCN- as a potential pharmacologic agent in diseases of chronic inflammation such as CF. Continuing studies will investigate the effect of nebulized SCN- during chronic infection of ENaC mice and littermates and changes in cytokine characteristics that may affect neutrophil trafficking. This work was supported by NIH grant RO1 HL084469 and a Cystic Fibrosis Foundation Research Grant.

PS 1002 Redox Cross-Talk between Myeloid-Derived Suppressor Cells and Dendritic Cells in Cancer: Accumulation of Oxygenated Lipid Droplets and Suppression of Antigen Cross-Presentation

V. A. Tyurin[1, 2], W. Cao[3], A. Amoscato[1, 2], J. Loomen[3], D. Gabrilovich[3] and V. E. Kagan[1, 2]. *[1]EOH, University of Pittsburgh, Pittsburgh, PA, [2]Center for Free Radic Antioxidant Health, University of Pittsburgh, Pittsburgh, PA and [3]The Wistar Institute Cancer Center, Philadelphia, PA.*

Accumulation of myeloid-derived suppressor cells (MDSCs), and expansion of immature dendritic cells (DCs) with aberrant cross-presentation – are important immunological abnormalities in cancer. While interactions between these cells are recognized as contributors to the failed anti-tumor immunity, their molecular mechanisms remain elusive. Excessive formation of lipid droplets (LDs) affects DC's immune functions. We show that DCs from tumor-bearing mice or treated with tumor explant supernatants (EL4, MC38) contained oxygenated neutral lipids: triglycerides (oxTAGs), free fatty acids (oxFFAs) and cholesterol esters (oxCE). LC-ESI-MS/MS revealed that oxTAGs were represented by a spectrum of truncated molecular species 39:1; 39:0; 41:1; 41:0; 43:2; 43:1; 43:0 and 45:2 with 9-oxo-nonanoic and 7-oxo-heptanoic acids. We hypothesized that MDSC – with their high ROS and myeloperoxidase activity - might be a source of oxygenated lipids. To test this, DCs were co-cultured with MDSC (Gr-1+,C57BL6 BM, pre-cultured with GM-CSF and deuterated linoleic acid, LA-d4) followed by purification of DCs from the mixture of cells. We found that LA-d4 was incorporated into

TAGs and several classes of phospholipids in MDSC and DC. Further, we demonstrated the transfer of LA-d4 from pre-loaded MDSC to DCs whereby increased amounts of oxFFAs and oxTAGs, including truncated TAGs, were identified. In contrast, no oxidized phospholipids were observed in either MDSC or DCs. By using a lipid-soluble radical azo-initiator, AMVN, we bolstered oxidation of LDs of LA-supplemented DCs and showed that accumulation of oxidized neutral lipids inhibited antigen cross-presentation. This suggests a novel role for oxidized lipids in immune responses – as regulators of antigen cross-presentation. Supported by NIH grants CA165065, U19 AI068021, and NIOSH OH008282.

PS 1003 Signaling Role of Cardiolipin Externalization in Elimination of Damaged Mitochondria in Lung Epithelial Cells

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Bacterial pneumonia triggers an exuberant host response characterized by excessive inflammation, oxidative stress, and epithelial cell damage culminating in the acute respiratory distress syndrome (ARDS). In ARDS, virulent bacterial pathogens directly damage host cells, activate innate immune responses, and trigger a burst in reactive oxygen species resulting in epithelial cell death. However, specific pathways involved in the evolution of epithelial injury with attendant release of novel damage signals remain elusive. We suggested that one critical damage signal is mitochondrial cardiolipin (CL). We found that mitophagy and reduced levels of distal mitochondrial protein markers were detectable in murine models of bacterial pneumonia. In addition, we demonstrated that early on after bacterial infection of murine lung epithelial cells (MLE15), CL transmigrates from the IMM to OMM; the unmasked externalized CL serves as a novel signal for mitophagy. A typical inducer of mitophagy, carbonyl cyanide m-chlorophenylhydrazone (CCCP) – acting as a protonophoric uncoupler - induced CL externalization to the OMM in MLE15 cells. This was evidenced by a robust decrease of the ratio of CL in the IMM and OMM from 18.6±0.3 in control MLE15 cells to 3.7±1.4 after treatment with CCCP. The externalization of CL was accompanied by activation of autophagy (LC3-I/II conversion) and decreased levels of mitochondrial marker proteins (TOM40, TIM23 and MnSOD) - consistent with mitophagy activation. CCCP-induced mitophagy in MLE was also confirmed by co-localization of mitochondria and lysosomes. Manipulations of CL levels or proteins involved in CL externalization (PLSR3) affected sensitivity of MLE to pro-mitophageal stimulation. These data are compatible with a hypothesis that externalized CL acts as an "eat-me" signal in depolarized mitochondria. Supported by NIH ES021069, U19 AI068021, NIOSH OH008282.

PS 1004 Resveratrol Protects against a Mouse Model of Multiple Sclerosis via Regulation of T Cell miRNA Expression

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Resveratrol is a plant-derived phytoestrogen with antioxidant, anti-carcinogenic and anti-inflammatory actions. Resveratrol has been extensively studied in cancer and in cardiovascular disease and has recently emerged as a potential therapeutic for the treatment of various inflammatory diseases. In this study, we evaluated the mechanistic effects of resveratrol on the pathogenesis of a mouse model of multiple sclerosis, experimental autoimmune encephalitis (EAE). We hypothesized that resveratrol protects against myelin oligodendrocyte peptide (MOG35-55)-induced neuroinflammation via regulation of T cell microRNA (miRNA) expression. Analysis of miRNA expression in CD4+ T cells from brains of EAE mice revealed significant up-regulation of miR-124,-128,-132,-138 and -155 with resveratrol treatment. Predicted and validated target genes were chosen for further examination based on gene ontology and functional analysis. miRNAs and target gene expression were validated by qRT-PCR. We found that resveratrol treatment leads to miRNA-mediated down regulation of genes essential for cell cycle progression (Rb1 and Cyclin D1) and growth and proliferation (p38 and Sphingosine Kinase 1) in CD4+ T cells. We also found that resveratrol decreases immune cell infiltration into the brain and impairs activation. While studies evaluating the effect of resveratrol on immune cells in the periphery exist, few studies have examined its effect on T cells in the target organ, the brain. Given the established neuroprotective effects and the rapidly growing anti-inflammatory properties, resveratrol is emerging as an ideal candidate for the treatment of multiple sclerosis and other neuroinflammatory diseases. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357)

PS 1005 CCR2 Regulates Proinflammatory Macrophage Migration into the Liver during Acetaminophen (APAP)-Induced Hepatotoxicity

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Inflammatory macrophages (MP) have been shown to play an important role in APAP-induced hepatotoxicity. Mechanisms mediating their accumulation in the liver have not been established. The C-C chemokine receptor, CCR2 has been shown to regulate inflammatory cell trafficking into injured tissues. In the present studies, we analyzed the phenotype of MP migrating into the liver following APAP intoxication and the role of CCR2 in this response. Mice were fasted overnight prior to administration of APAP (300 mg/kg, i.p.) or PBS. Liver, spleen and bone marrow (BM) were collected 24-96 h later and analyzed by flow cytometry. APAP caused a time-dependent increase in CD11b+Ly6Chi pro-inflammatory MP in the liver, but a transient decrease in CD11b+Ly6Clo anti-inflammatory/wound repair MP. Loss of CCR2 significantly reduced CD11b+Ly6Chi cells accumulating in the liver in response to APAP. We previously showed that the spleen is a source of hepatic inflammatory MP. Following APAP administration, increases in CD11b+Ly6Chi monocytes were observed in the spleen, with no effect on CD11b+Ly6Clo monocytes. Loss of CCR2 reduced numbers of pro-inflammatory monocytes in the spleen. In contrast, no differences were observed in monocyte subsets in the BM between wild type (WT) and CCR2-/- mice. We also identified a subpopulation of CD11b+Ly6G+Ly6C+ myeloid derived suppressor cells (MDSC) in the liver and spleen of WT mice, which decreased 72 and 96 h after APAP. In contrast, in CCR2-/- mice, MDSC increased within 24 h of APAP, remaining elevated for 72-96 h. Taken together, these results indicate that CCR2 plays a role in the emigration of CD11b+Ly6Chi pro-inflammatory MP into the liver from BM and spleen following APAP hepatotoxicity. Moreover, loss of CCR2 results in increased MDSC in the liver which may contribute to tissue repair. Supported by NIH ES007148, ES004738, CA132624, AR055073, and ES005022.

PS 1006 Collagen Synthesis and Degradation Are Enhanced in Chronic Bacterial-Induced Prostatic Inflammation

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Benign prostatic hyperplasia (BPH) is a common disorder in aging men. Prostatic inflammation-induced fibrosis is suggested to be a major contributor of BPH etiology. Collagen deposition was increased in our previously described mouse model of bacterial prostatic inflammation. The goal of this study is to investigate the synthesis and degradation of newly synthesized collagen in bacterial-induced prostatic inflammation.

Adult C3H/HeOuJ male mice were transurethrally instilled 200ul of 2x106/ml of uropathogenic *E. coli* 1677 or saline to induce prostatic inflammation. Prostates were harvested to perform immunohistology at selected timepoint following infection. To measure collagen synthesis, mice were injected with 15μCi of 3H-proline on day 0, 5, 12, 19, 26 post-instillation. Prostates were harvested 2 days after 3H-proline injection. To measure collagen degradation, mice were injected with 15μCi of 3H-proline every 2 days for 28 days. Prostates were harvested on day 0, 4, 8, 15, 22, 29, 36, 57, 78, 113 after the last injection of 3H-proline. The incorporation of 3H-hydroxyproline was determined by measuring 3H radioactivity using a liquid scintillation counter.

Collagen synthesis was significantly increased in the infected prostates between 5 and 7 days, and 12 and 14 days post-instillation. Immunostaining to determine collagen-producing cell revealed that there were abundant CD45+Vimentin+ fibrocytes coexpressing with the collagen synthesizing enzyme prolyl hydroxylase in the infected prostates on day 7 after infection. The newly synthesized collagen in prostatic inflammation was less stable than that in the saline-instilled prostates. The half-life of collagen in the saline prostates was 18.6 days while collagen in the infected prostates had a half-life of 13.7 days.

Our study showed that increased collagen turnover and the presence of fibrocytes characterize prostatic fibrosis in bacterial-induced inflammation. This finding provides a basis for future study to elucidate the cellular and molecular mechanisms of collagen deposition in inflammation-associated prostatic diseases.

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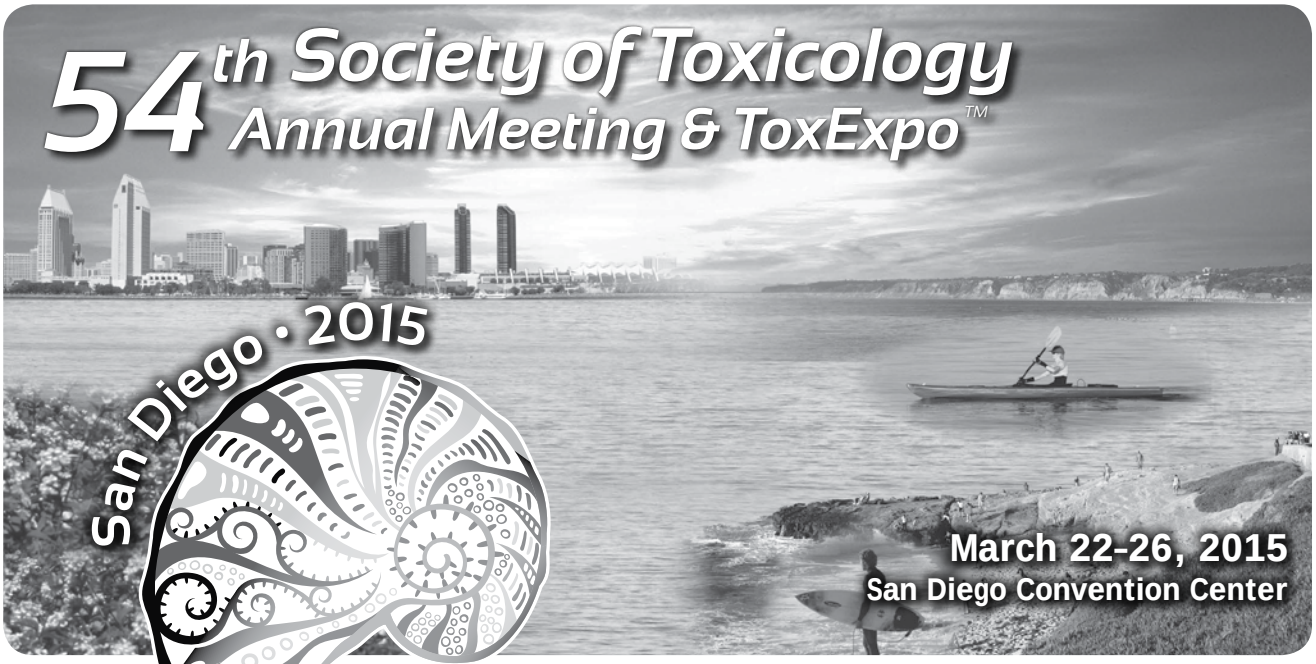
ISSN 1096-6080
Volume 138, Issue 1
March 2014

www.toxsci.oxfordjournals.org



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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 53rd Annual Meeting of the Society of Toxicology, held at the Phoenix Convention Center, March 23–27, 2014.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 627.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 655.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

NOTE: Abstract numbers including a lower-case letter were programmed during second submission phase.

Scientific Session Types:

- EC Education-Career Development Sessions
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